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Synthetic Studies on Serine and Threonine β-Lactones and the Design and Synthesis of Pyrophosphate Mimics.

BY

Elaref S. Ratemi ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

DEPARTMENT OF CHEMISTRY

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To my parents, my wife Fuzia and son Zakaria

ABSTRACT

A synthetic methodology for the preparation of various β-amino-L-alanine derivatives was developed. The process involved the regiospecific ring opening of *N*-Cbz-L-serine β-lactone (**7**) using *N*-silylamine reagents. A variety of *N*-silylamines attacked **7** in acetonitrile with alkyl-oxygen cleavage to give good yields (45-88 %) of the corresponding β-amino L-alanine derivatives **24-31**. The reaction conditions are mild and the method is applicable to trialkylsilyl derivatives of ammonia as well as of primary, secondary and heterocyclic amines. The ring opening of **7** with aluminum•amine complexes was also regiospecific, but it proceeded *via* acyl-oxygen cleavage to give the corresponding L-serinamides in excellent yields. Preliminary investigations of the mode of ring opening of *N*-(*o*-nitrophenyl)sulfenyl-L-threonine β-lactone (**11**), by the same reagents mentioned above, revealed that for both cases acyl-oxygen cleavage was the preferred reaction giving the corresponding L-threoninamides.

Chaetomellic acid A (**44**), a potent inhibitor of protein farnesyltransferase (PFTase), was prepared in 78 % overall yield by a facile, two step, stereospecific synthesis using commercially available starting materials. The approach was based on the tandem vicinal difunctionalization of dimethyl acetylenedicarboxylate (DMAD) through a conjugate addition/enolate trapping sequence. Thus, the addition of the organocuprate reagent, derived from magnesium chloride and CuBr•Me_2S to DMAD in THF containing HMPA, followed by trapping of the resulting copper enolate with methyl iodide gave chaetomellic acid A methyl ester (**42a**). Hydrolysis of **42a** with lithium hydroxide gave a quantitative yield of **44** which cyclized rapidly to the corresponding anhydride **45** in the presence of acid. This represents the simplest and most efficient approach to chaetomellic acid A reported thus far.

This synthetic strategy was successfully applied to the synthesis of various chaetomellic acid A analogues, some of which were found to be potent inhibitors of PFTase and protein geranylgeranyltransferase (PGGTase). Derivative **57**, containing a side chain with two carbons shorter than the side chain of chaetomellic acid A, was found to be a more potent inhibitor of yeast PFTase than chaetomellic acid A itself. Compound **58**, an analogue wherein the tetradecyl group of **44** was replaced by a farnesyl moiety, was 7-fold more potent than **44** as an inhibitor of PFTase from yeast and displays a 100:1 selectivity for this enzyme relative to yeast PGGTase. In contrast, analogue **59**, which contains a geranylgeranyl side chain, was a potent inhibitor of PGGTase and showed a 10:1 selectivity for this enzyme versus PFTase.

Synthetic studies directed towards the preparation of potential inhibitors of transglycosylase, an enzyme responsible for the formation of the polysaccharide backbone of bacterial peptidoglycan, were undertaken. The design of the synthetic targets was based on the structural features of both the natural inhibitor, moenomycin A, and the natural substrate, peptidoglycan monomer (PGM). (*Z*)-2-β-D-Glucopyranosyloxymethyl-3-tetradecylbutenedioic acid, disodium salt (**100**), designed to mimic an active moenomycin A degradation product, was synthesized by a convergent approach in 21% overall yield. The *N*-acetylglucosamine and chitobiose analogues (Type **C** molecules) of **100** were designed to mimic PGM. Synthetic studies towards the former derivative were carried out using the glycosyl acceptor dimethyl (*Z*)-2-hydroxymethyl-3-tetradecylbutenedioate (**89**), which was made *via* the conjugate addition approach mentioned above, and the glycosyl donors 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)[2,1-*d*]-Δ^2oxazoline (**102**) and 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyltrichloro-acetimidate (**108**). The glycosylation reactions of the glycosyl acceptor **89** and the analogous glycosyl donors oxazoline **104** and imidate **111**, which were both prepared from peracetylated chitobiose **103**, were also examined.

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I would like to thank my supervisor, Professor John C. Vederas, for his excellent guidance, support, and encouragement throughout my studies. Professor C. Dale Poulter is greatly acknowledged for his collaborative efforts. I thank all the members in our research group for their help at various points during this work, in particular Dr. Jane M. Taylor for her encouragement and help. I am indebted to Drs. Mark Andrews and John McKendrick for proof-reading my thesis and for helpful discussions. Drs. Joanna Harris, David Brown and Kurt Wagschal are also acknowledged for proof-reading parts of this manuscript. The staff of spectral and analytical services in the Department of Chemistry are acknowledged for their assistance in characterizing compounds. Finally, I would like to thank my wife Fuzia for her care, understanding, and encouragement during this work.

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LIST OF ABBREVIATIONS

[α]	specific rotation
Ac	acetyl
AIBN	2,2'-azobisisobutyronitrile
Ala	alanine
APT	attached proton test
Ar	aryl
Bn	benzyl
Boc	*tert*-butoxycarbonyl
BOM	benzyloxymethyl
bp	boiling point
br	broad
i-Bu	isobutyl
n-Bu	butyl
calcd	calculated
Cbz	benzyloxycarbonyl
CI	chemical ionization
CoA	coenzyme A
COSY	correlation spectroscopy
δ	chemical shift in parts per million downfield from tetramethylsilane
d	doublet
DABCO	1,4-diazabicyclo[2.2.2]octane
m-DAP	*meso*-diaminopimelate
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N,N'*-dicyclohexylcarbodiimide

DMAD	dimethyl acetylenedicarboxylate
DME	1,2-dimethoxyethane
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
EDCI	1-ethyl-3-[3-(dimethyl amino)propyl]-carbodimide
EI	electron impact
ES	electrospray
Et	ethyl
FAB	fast atom bombardment
FPP	farnesyl pyrophosphate
Glc	glucose
GlcNAc	*N*-acetylglucosamine
Glu	glutamic acid
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
HMPA	hexamethylphosphoric triamide
HMQC	heteronuclear multiple quantum coherence
HRMS	high-resolution mass spectrum
IR	infrared
J	coupling constant
LHMDS	lithium hexamethyldisilazane
m	multiplet
m/z	mass to charge ratio
Me	methyl
MHz	megahertz
min	minute(s)
mol	mole(s)

MOM	methoxymethyl
mp	melting point
MS	mass spectrometry
MurNAc	*N*-acetylmuramic acid
NAD	nicotinamide adenine dinucleotide
NADH	reduced NAD
NBS	*N*-bromosuccinimide
NMR	nuclear magnetic resonance
nOe	nuclear Overhauser effect
Nu	nucleophile
PBP	penicillin binding protein
PFTase	protein farnesyltransferase
PGGTase	protein geranylgeranyltransferase
PGM	peptidoglycan monomer
Ph	phenyl
Phth	phthalimido
P_i	phosphate
pp_i	pyrophosphate
ppm	parts per million
pyr	pyridine
q	quartet
qn	quintet
R_f	retention factor
RP	reverse phase
R_t	retention time
rt	room temperature

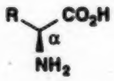
s	singlet
SEM	trimethylsilylethoxymethyl
t	triplet
TBAF	tetrabutylammonium fluoride
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl, tetramethylsilane
TMSI	trimethylsilyl iodide
TMSOTf	trimethylsilyl triflate
Tr	triphenylmethyl (trityl)
Ts	*p*-toluenesulfonyl
UDP	uridine diphosphate
UMP	uridine monophosphate
UTP	uridine triphosphate
UV	ultraviolet

CHAPTER 1 α,β-Diamino Acids *via* Ring Opening of α-Amino β-Lactones

INTRODUCTION

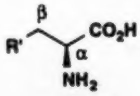
1. General.

α-Amino acids constitute a large and diverse group of biologically important molecules. Of this group of molecules, the 20 common L-α-amino acids are the constituents of proteins and peptides indispensable for life. The biological importance[1-5] and synthetic utility[6-16] of α-amino acids has prompted the recent development of numerous methods for their stereospecific synthesis.[17-34] Many α-amino acids can be further classified as β-substituted alanines and most have at least one asymmetric carbon. β-Substituted alanines occur in higher plants, or as constituents of microbial peptides possessing antibiotic or antitumor activity.[35-38]



α-amino acid

R = CH_3, alanine



β-substituted alanine

R' = OH, serine

The strategies employed for amino acid preparation can be divided into three broad categories: resolution (either classical or enzymatic), asymmetric synthesis and 'chiral pool' elaboration. The latter semi-synthetic approach is perhaps the most attractive because of the commercial availability of the proteinogenic α-amino acids with high optical purity. For example, in the synthesis of β-substituted alanines, the amino acid serine is an attractive starting material since both enantiomers are commercially available in pure form, and the hydroxyl group at the β-position provides access for further transformation.

2. Approaches to β-substituted α-amino acids.

Approaches involving activation of the hydroxyl group of serine followed by nucleophilic displacement (path a) suffer from low yields and often loss of stereochemistry at the α-position. This can be a result of β-elimination (path b) followed by re-addition of the nucleophile in a conjugate addition manner or can be as a result of direct epimerization due to deprotonation protonation (Figure 1).[39-49]

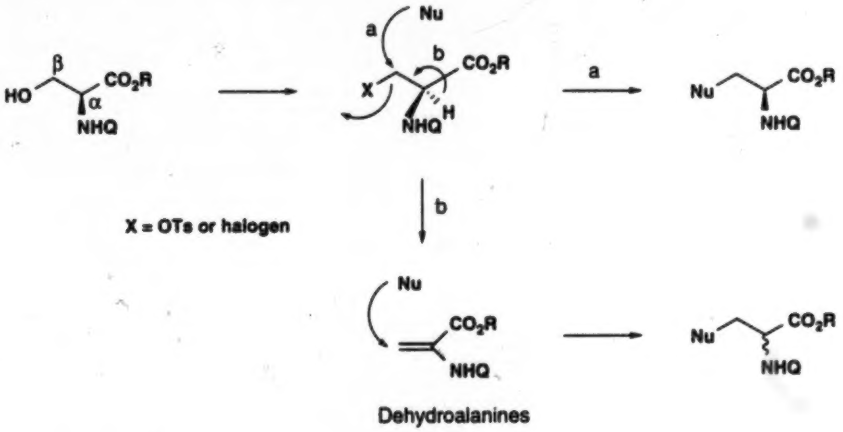


Figure 1. β-Substituted alanines *via* nucleophilic substitution on alanine derivatives bearing a leaving group at the β-position.

In order to minimize the competing β-elimination process, Baldwin and coworkers carried out the β-substitution in an intramolecular manner during the synthesis of β-amino alanine derivatives.[52] Thus, α-*N*-*tert*-butoxycarbonyl-β-amino alanine **4** (Q = Boc) was prepared from β-chloroalanine **1**, *via* isoxazolidin-5-one **3**, in 55 % yield over 4 steps (Figure 2).[50-52] Although this strategy gives clean products, more steps are needed, especially when one considers the fact that β-chloroalanine **1** must first be synthesized from L-serine.

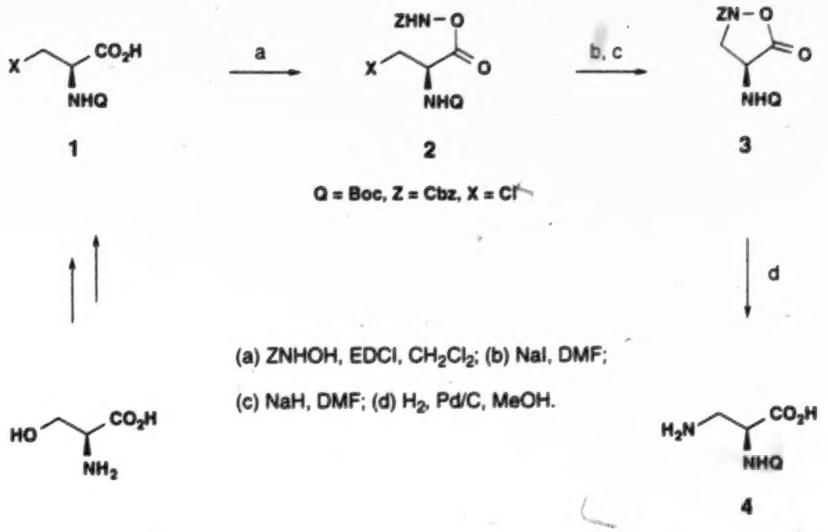
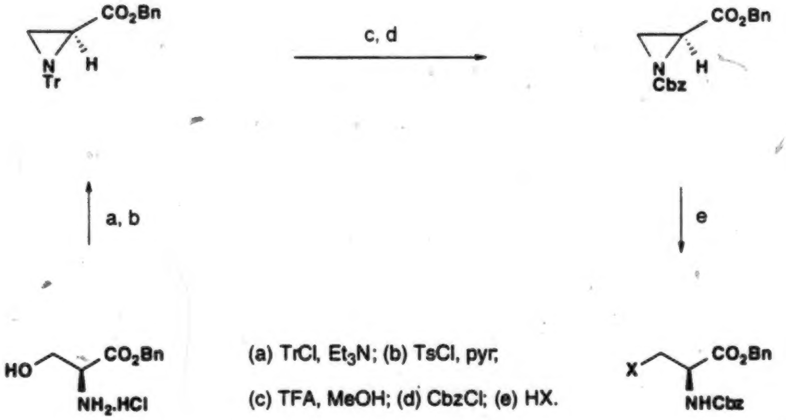

1
a
2
b, c
3
Q = Boc, Z = Cbz, X = Cl
d
(a) ZNHOH, EDCI, CH2Cl2; (b) NaI, DMF;
(c) NaH, DMF; (d) H2, Pd/C, MeOH.
4

Figure 2. β-Amino alanines *via* isoxazolidin-5-one (**3**).

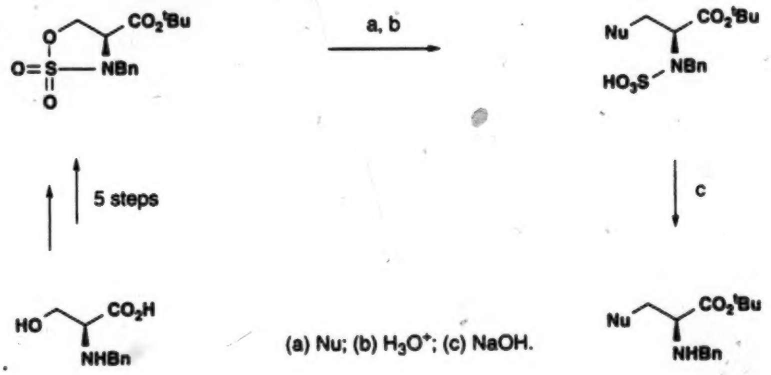
A similar approach to the formation of β-substituted alanines is based on the ring opening of enantiomerically pure aziridine-2-carboxylates (Figure 3).[53,54] Nucleophiles such as amines,[55-57] thiols,[58,59] thio-carboxylic acids,[60] alcohols,[58,61] carboxylic acids[62] and halides[63] have been found to attack aziridines to give β-substituted alanines. This process has also been extended to include carbon nucleophiles.[64-66] Even though this approach is attractive, in that it leads to the formation of stereochemically pure β-substituted alanines, it suffers from the major disadvantage that the aziridine required must be synthesized over several steps. Furthermore, $BF_3 \cdot Et_2O$ catalysis is often required during the ring opening process and certain nucleophiles (e.g. amines[54] and carbon-based nucleophiles[66]) appear to be less efficient for ring opening.


CO$_2$Bn
N
Tr
H
c, d
CO$_2$Bn
N
Cbz
H
a, b
e
HO
CO$_2$Bn
NH$_2$.HCl
X
CO$_2$Bn
NHCbz

(a) TrCl, Et$_3$N; (b) TsCl, pyr; (c) TFA, MeOH; (d) CbzCl; (e) HX.

Figure 3. β-Substituted alanines *via* the ring opening of an aziridine carboxylate.

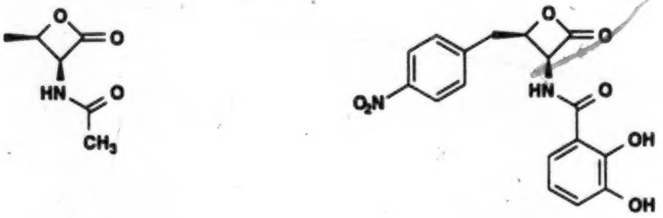
The attractive exploitation of vicinal diol cyclic sulphates as reactive epoxide equivalents by Sharpless[67,68] prompted Baldwin and co-workers to use the analogous cyclic sulphamidates as synthetic precursors for β-substituted α-amino acids (Figure 4).[69,70] The ring opening step is efficient with a variety of nucleophiles, however, the synthesis is still considered lengthy since the sulphamidate has to be prepared from *N*-benzyl serine over 5 steps.[69]


CO$_2$^{t}Bu
O
O=S—NBn
O
a, b
Nu
CO$_2$^{t}Bu
NBn
HO$_3$S
5 steps
c
HO
CO$_2$H
NHBn
Nu
CO$_2$^{t}Bu
NHBn

(a) Nu; (b) H$_3$O$^+$; (c) NaOH.

Figure 4. Cyclic sulphamidates as synthetic precursors for β-substituted α-amino acids.

The shortest, most attractive, approach to the synthesis of stereochemically pure β-substituted α-amino acids has been developed by Vederas and co-workers.[71] This methodology is based on the ring opening of α-amino β-lactones[71-78] and involves minimal derivatization of the starting amino acid (see section **4**). α-Amino β-lactones are not only important synthetic intermediates but are also of great interest, since some of them display interesting antibiotic activity. For example, SQ 26,517[79,80] and obafluorin[81-83] (Figure 5) are among the naturally occurring β-lactones produced by microbes that exhibit antibiotic activity. A general approach for the total synthesis of these compounds has also been achieved.[84-87]



SQ 26,517 (+)-obafluorin

Figure 5. Naturally occurring β-lactone antibiotics.

3. Approaches to the synthesis of α-amino β-lactones.

There are many approaches to construct a β-lactone ring,[88-91] but the number of methods to synthesize α-amino β-lactones is limited. The most common approach is the cyclization of amine-protected β-hydroxy α-amino acids (L-serine and L-threonine being the most common).[71,72,76-78]

The synthetically useful serine β-lactones **7** and **8** have been prepared *via* modified Mitsunobu conditions using a preformed complex of dimethyl azodicarboxylate and triphenyl phosphine at low (-78 °C) temperature. The Mitsunobu ring closure of **5** or

6 proceeds by hydroxyl group activation (HGA) with subsequent loss of the oxygen atom at C-3 and inversion of configuration at that site to give **7** or **8** (Figure 6).[71,77,78]

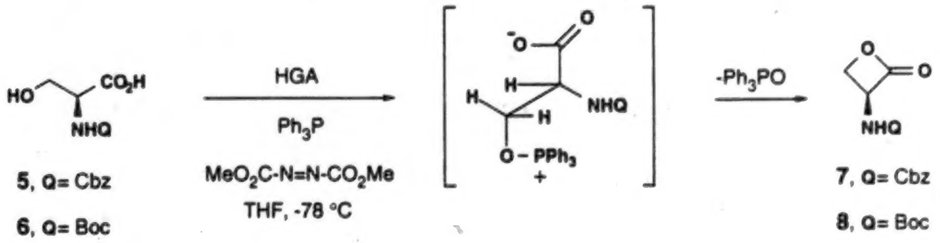

HO
CO2H
NHQ
5, Q= Cbz
6, Q= Boc
HGA
Ph3P
MeO2C-N=N-CO2Me
THF, -78 °C
O
O
H
H
NHQ
H
O - PPh3
+
-Ph3PO
O
O
NHQ
7, Q= Cbz
8, Q= Boc

Figure 6. Serine β-lactones from β-hydroxy α-amino acids.

Evidence for inversion at C-3 came from deuterium labeling studies, while evidence for cyclization by hydroxyl group activation (HGA) came from ^{18}O labeling experiments. For example, when *N*-benzloxycarbonyl L-serine **5a** was cyclized, all of the ^{18}O label was retained in **7a**, whereas cyclization of **5b** proceeded with complete loss of ^{18}O to give unlabelled lactone **7** (Figure 7).[72]

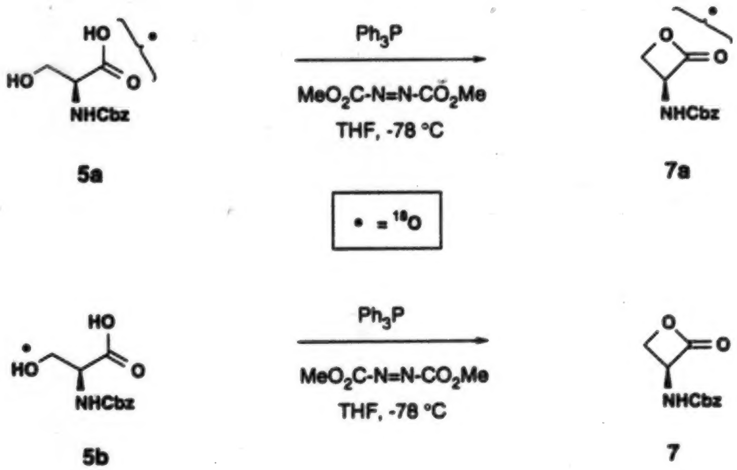

HO
HO
O
NHCbz
5a
Ph3P
MeO2C-N=N-CO2Me
THF, -78 °C
O
O
NHCbz
7a
• = 18O
HO
HO
O
NHCbz
5b
Ph3P
MeO2C-N=N-CO2Me
THF, -78 °C
O
O
NHCbz
7

Figure 7. Modes of Mitsunobu cyclization of ^{18}O-labeled serines.

However, Mitsunobu conditions can not be applied to the other most common β-hydroxy amino acid, threonine, because rapid stereospecific decarboxylative *anti* elimination intervenes (Figure 8).[76] Apparently, the methyl group at the β-position hinders the nucleophilic displacement by the carboxyl group in the phosphonium intermediate and allows the elimination process to dominate.

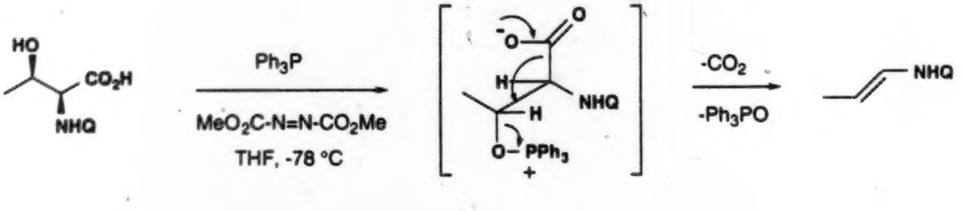


Figure 8. Decarboxylative elimination of *N*-alkyloxycarbonyl L-threonine under low temperature Mitsunobu conditions.

This problem can be circumvented by carboxyl group activation (CGA), however, the yields are low if the protecting group is capable of forming azlactone **9** (Figure 9).[76, 91-93]

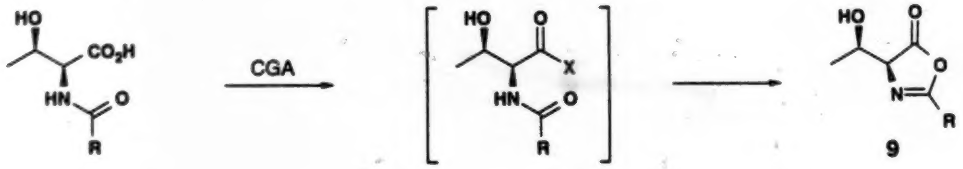
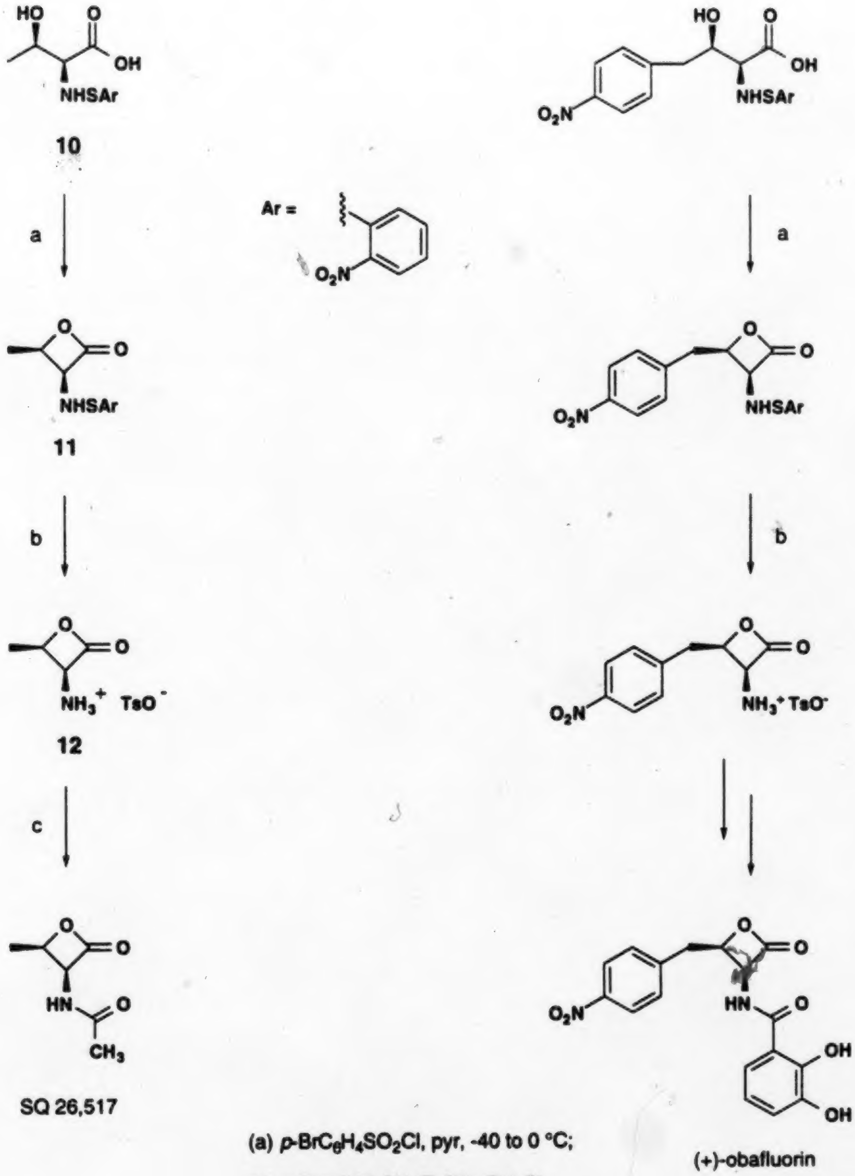


Figure 9. Azlactone formation.

The use of the *o*-nitrophenylsulfenyl protecting group[94] avoids this problem and threonine β-lactone was formed in good yield *via* carboxyl group activation. This method was used successfully in the synthesis of SQ 26,517 and (+)-obafluorin (Figure 10).[84]



HO
O
OH
NHSAr
10
a
Ar =
O_2N
11
b
NH_3^+ TsO$^-$
12
c
HN
O
CH_3
SQ 26,517
HNSAr
(+)-obafluorin
OH
OH

(a) p-$BrC_6H_4SO_2Cl$, pyr, -40 to 0 °C;

(b) p-$CH_3C_6H_4SH$, TsOH, CH_2Cl_2;

(c) CH_3COCl, pyr, -10 to 20 °C.

Figure 10. Synthesis of antibiotics SQ 26,517 and (+)-obafluorin *via* β-substituted β-lactones.

4. α-Amino β-lactones as synthetic intermediates.

The reactivity of the β-lactone ring is unique due to the high angle strain (23 kcal mol^{-1}).[95,96] Another important feature of β-lactone reactivity is that nucleophilic attack can proceed at either the methylene carbon, with alkyl-oxygen cleavage (Figure 11, path **a**), or at the carbonyl carbon with acyl-oxygen cleavage (Figure 11, path **b**).

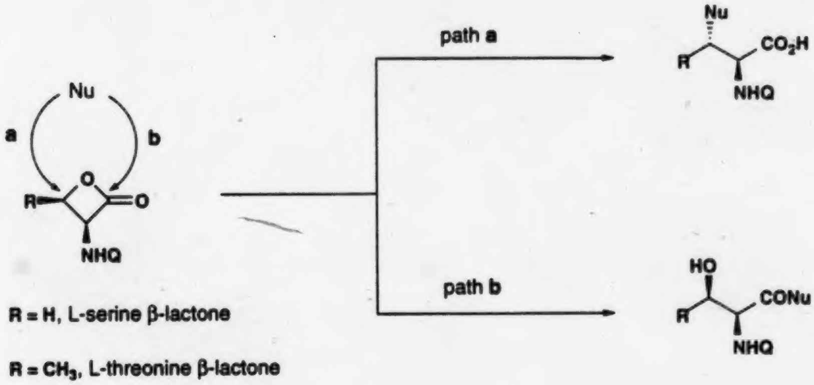


Figure 11. General pathways for nucleophilic ring opening of α-amino β-lactones.

4.1 β-Substituted α-amino acids *via* α-amino β-lactones.

The ring opening of α-amino β-lactones with alkyl-oxygen cleavage (Figure 11, path **a**) is an attractive route for the synthesis of optically pure α-amino acids. Studies have shown that the outcome of the reaction is governed by the nature of the nucleophile and the reaction conditions. For serine β-lactones,[71-78,97,98] "hard" nucleophiles (e.g. hydroxide, alkoxide and organolithium) attack the carbonyl carbon, whereas "softer" nucleophiles (e.g. carboxylate, thiolate) tend to target the β-position. Treatment of *N*-alkyloxycarbonyl (e.g. Boc or Cbz) serine β-lactones, **7** and **8**, with a variety of halogen, carbon or heteroatom nucleophiles (Y:) results in a number of novel β-substituted α-amino acids with no loss of stereochemical integrity (Figure 12). If the protecting group (Q) is *tert*-butyloxycarbonyl (Boc) as in **8**, then treatment with non-nucleophilic acids (e.g. TsOH) provides salts such as **13**. These salts react similarly with various nucleophiles to allow direct access to unprotected β-substituted alanines.[74] Initial studies on threonine β-lactones have shown that ring opening tends to proceed by attack at the carbonyl carbon except with certain nucleophiles (e.g. thiourea, halides) which attack at the β-carbon.[76]

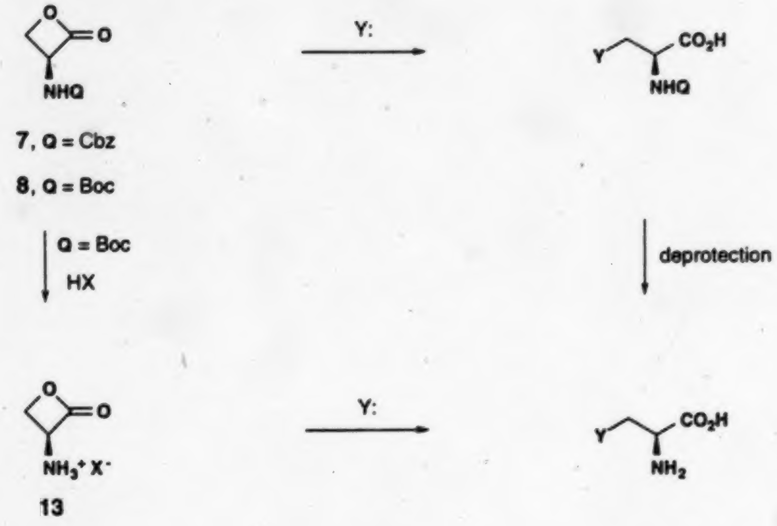


Figure 12. Synthesis of β-substituted α-amino acids by nucleophilic ring opening of protected and deprotected L-serine β-lactones.

4.2. β-Amino alanines.

Derivatives of β-amino L-alanine ((2*S*)-2,3-diaminopropanoic acid) occur in nature both as free amino acids and as constituents of peptides with antibiotic and antitumor activity.[99,100] Many such compounds contain heterocyclic rings at the β-carbon and display neurotoxic effects.[99-101] Synthetic derivatives of β-amino alanine and peptides containing them have also proved useful for enzyme inhibition studies[102] and for the construction of metal chelating peptides.[103] Of the methods[104] presented above for the synthesis of such optically active compounds, the ring opening of serine β-lactones with nitrogen nucleophiles is very attractive because it avoids problems such as lack of sterochemical control[40-49,105] and lengthy synthesis[50-57] that are usually associated with the approaches discussed above.

Thus, if the ring opening reaction proceeds by regiospecific attack of nitrogen nucleophiles at the β-position (Figure 11, path **a**), then β-amino alanines are produced in regio- and stereo-chemically pure form.[75,77,78] However, the situation is less straightforward with nitrogen nucleophiles and the outcome of the reaction is usually dependent on the nature of both the reactants and the solvent. For example, ammonia in THF attacks the β-position of **7** to give the protected β-amino alanine **14**, whereas the same nucleophile in CH_3CN attacks the carbonyl carbon to give serine amide **15** (Figure 13).[75] On the other hand, CH_3CN enhances β-attack by ammonia when *N*-Boc lactone **8** is used.[106]

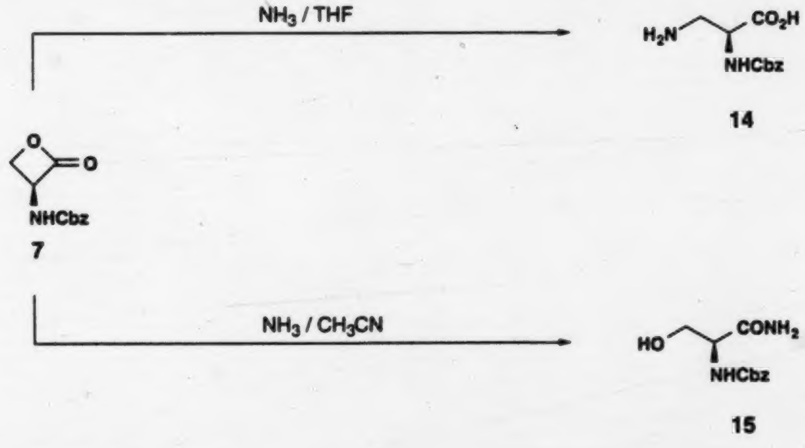


Figure 13. Effect of solvent on the mode of addition to *N*-Cbz-L-serine β-lactones.

The undesired acyl-oxygen cleavage of serine β-lactones is sometimes the sole mode of addition of nitrogen nucleophiles.[71,107,108] In certain cases, especially with β-propiolactones, the situation is even less desirable with a mixture of products arising from both alkyl-oxygen and acyl-oxygen cleavage being obtained.[109] Hence, more reliable control over regiochemistry of attack is clearly desirable.

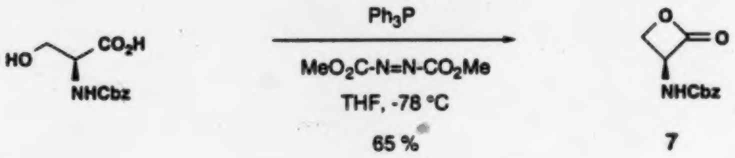
The following section describes the ring opening reactions of optically pure *N*-Cbz-L-serine β-lactone **7** by aluminum amine and *N*-silyl amine reagents to afford good yields of L-serinamides and β-amino-L-alanine derivatives, respectively. Initial studies on (*o*-nitrophenyl)sulfenyl L-threonine β-lactone **11** will also be presented.

RESULTS AND DISCUSSION

I Studies on *N*-Cbz-L-Serine β-Lactone 7.

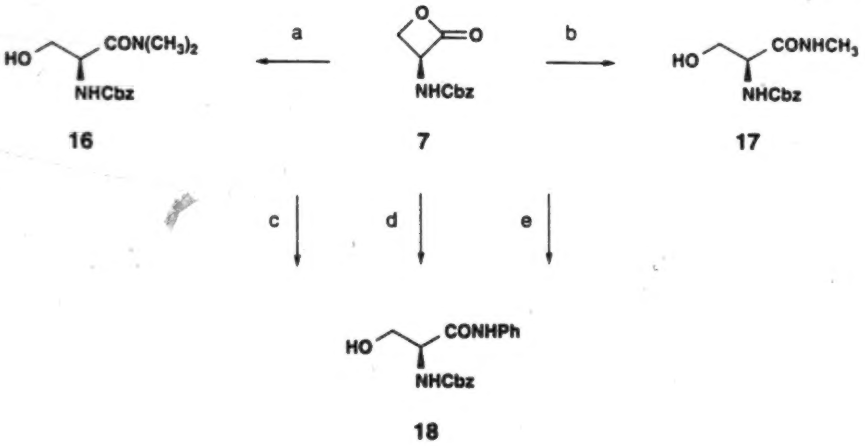
1. Ring Opening With Aluminum•Amine Complexes.

To accomplish the following studies, multi-gram quantities of *N*-Cbz-L-serine β-lactone **7** were required. β-Lactone **7** was conveniently prepared by the well established methodology of Vederas (Scheme 1).[73] The choice of the benzyloxycarbonyl (Cbz) moiety as the nitrogen protecting group of the serine β-lactone was based on the fact that this group is compatible with the widest range of conditions, it is easy to remove and its use is well precedented in amino acid chemistry.



Scheme 1.

Since Lewis acid or metal ion catalysis can direct nucleophilic attack on *N*-protected serine β-lactones[73] and β-propiolactone,[107] aluminum-amine reagents[108] appeared likely to cleave **7** with high regiospecificity. As seen in Scheme 2, reagents derived from diethylaluminum chloride (Et_2AlCl) and an amine (or amine hydrochloride) reacted smoothly and regiospecifically at the carbonyl carbon with acyl-oxygen cleavage to afford high isolated yields of the corresponding L-serinamides. Trimethylaluminum could also be used in place of diethylaluminum chloride to give **18**, but in lower yield (75 %). When the reaction of phenylamine with serine β-lactone **7** was performed in the presence of $AlCl_3$, serinamide **18** was isolated in 92 % isolated yield (Scheme 2).



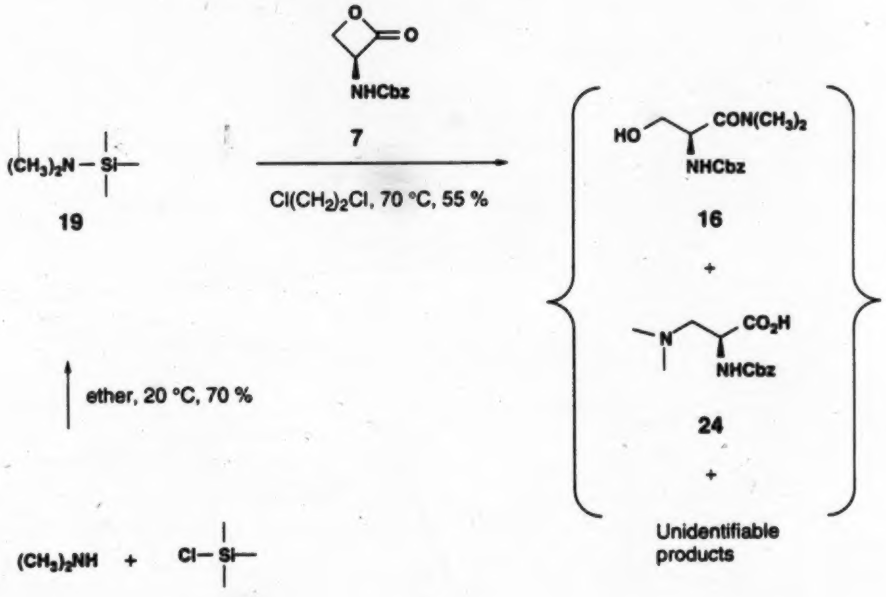
(a) $Et_2AlCl{\cdot}HN(CH_3)_2$, CH_2Cl_2, 0 °C, 84 %; (b) $Et_2AlCl{\cdot}H_2NCH_3$, CH_2Cl_2, 0 °C, 81 %; (c) $Et_2AlCl{\cdot}H_2NPh$, CH_2Cl_2, 0 °C, 89 %; (d) $(CH_3)_3Al{\cdot}H_2NPh$, CH_2Cl_2, 0 °C, 75 % ; (e) $AlCl_3$, H_2NPh, CH_2Cl_2, 0 °C, 92 %.

Scheme 2.

This mode of ring opening by these reagents was not actually surprising in light of their efficiency in the conversion of esters and γ-lactones to amides,[109] thioesters[110] and nitriles.[111] Although the serinamides were not the desired products, their preparation by this route is mild and gives high yields of easily isolated pure products. It may also prove useful in those cases where hydroxyl group protection / deprotection in peptide synthesis should be avoided. The above results suggest that the amine moiety is quite labile when it is bound to aluminum and upon coordination of the carbonyl oxygen to aluminum, the amino group may be displaced to then attack the electron-deficient carbonyl carbon.

2. Ring Opening With *N*-Silylamine Reagents.

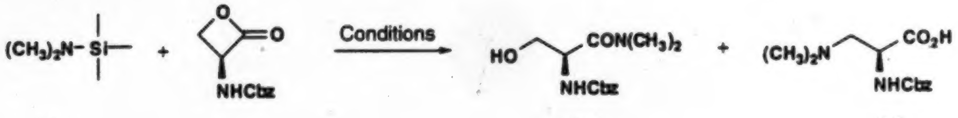
Compounds containing a silicon-nitrogen bond are known to react with polar double bonds. Itoh and co-workers reported that trimethylsilyldialkyl amines react with β-propiolactone preferentially with alkyl-oxygen cleavage to give trimethylsilyl esters of β-aminopropionates.[112] Thus, we prepared a series of silylamines **19-23** (Table 2) to study their reaction with β-lactone **7**. The reaction of *N,N*-dimethyl-*N*-(trimethylsilyl)-amine (**19**) with **7** under the same conditions reported for the ring opening of β-propiolactone[112] proceeded by both acyl-oxygen and alkyl-oxygen cleavage to give serinamide **16** and amino acid **24**, respectively, as a mixture (40:60) in 55 % yield. Some decomposition of **7** also seems to have occurred under these relatively harsh conditions (70 °C), contributing to the low yield.


O
O
NHCbz
7
Cl(CH2)2Cl, 70 °C, 55 %
(CH3)2N – Si –
19
HO
CON(CH3)2
NHCbz
16
+
N
CO2H
NHCbz
24
+
Unidentifiable products
ether, 20 °C, 70 %
(CH3)2NH + Cl – Si –

Scheme 3.

When different reaction conditions were examined, the mode of ring opening was found to be solvent dependent (Table 1). The use of acetonitrile at room temperature gave the best selectivity for amino acid formation *via* alkyl-oxygen cleavage and also afforded an excellent overall yield (entry 5, Table 1). In THF, the selectivity was reasonable and the overall yield was also excellent (entry 4, Table 1). In $Cl(CH_2)_2Cl$ at 20 °C (entry 3, Table 1) the selectivity was better than at 70 °C and no decomposition of β-lactone **7** was observed. The cause of these solvent effects is presently unknown, but it may be due to enhanced stabilization of charge separation in the transition state by a more polar aprotic medium such as acetonitrile.

Table 1. Solvent effects on the reaction of *N,N*-dimethyl-*N*-(trimethylsilyl)amine **19** with serine β-lactone **7**.

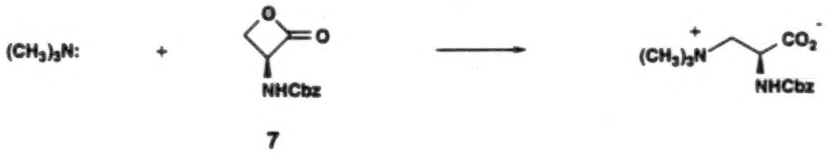


19 **7** **16** **24**

Entry	Conditions	Product Ratio		Yield (%)
		Amide	Amino Acid	
1	$CHCl_3$, 20 °C, 3 h	80	20	88
2	CH_2Cl_2, 20 °C, 1 h	64	36	85
3	$Cl(CH_2)_2Cl$, 20 °C, 1 h	35	65	90
4	THF, 20 °C, 8 h	18	82	92
5	CH_3CN, 20 °C, 4 h	5	95	95

A variety of other *N*-trimethylsilyl amines reacted analogously with β-lactone **7** in acetonitrile to give good yields of the corresponding β-amino L-alanine derivatives (Table 2). The reaction conditions were mild and simple extraction of the aqueous layer during workup allowed facile isolation of the optically pure amino acid uncontaminated by any minor amounts of amide which may have been formed. Medium pressure liquid chromatography (MPLC) of the crude product on a reverse phase (C-8) column rapidly gives analytically pure material.

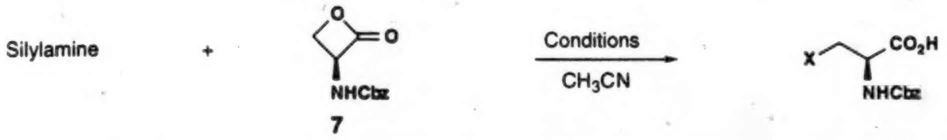
This method is applicable to trialkylsilyl derivatives of ammonia as well as of primary, secondary and heterocyclic amines. Parent (unsilylated) tertiary amines (e.g. trimethylamine) have previously been shown to react exclusively at the β-carbon of **7** to give the β-substituted amino acids as internal salts (Scheme 4).[71]



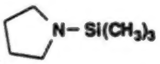
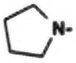
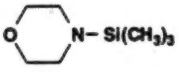
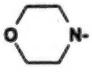
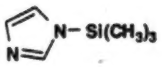
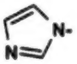
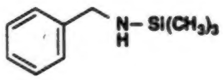
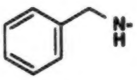
Scheme 4.

The fact that the bond cleavage of **7** by trimethylsilyl amines occurred at the same position as that by tertiary amines suggests that the reaction between *N*-silylamines **19** and **7** may also involve a similar intermediate such as **I** (Scheme 5). In other words, the nucleophilic attack by the nitrogen atom in *N*-silylamines might be important, as in tertiary amines. A possible mechanism for the ring opening by *N*-silylamines is shown in Scheme 5. Further kinetic studies of these addition reactions would be necessary to support such a mechanism.

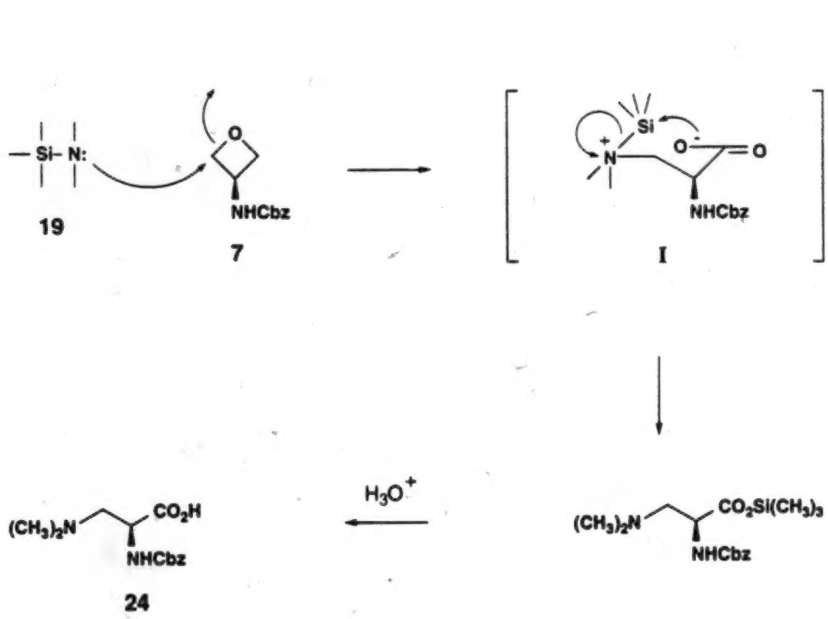
Table 2. β-Amino alanines *via* ring opening of L-serine β-lactone **7** by *N*-silylamines.



Silylamine
+
O
O
NHCbz
7
Conditions
CH3CN
X
CO2H
NHCbz

Silylamine	Conditions	X	Product	Yield (%)
$(CH_3)_2N\text{-}Si(CH_3)_3$ **19**	20 °C, 2 h	$(CH_3)_2N$-	**24**	88
$(C_2H_5)_2N\text{-}Si(CH_3)_3$ **20**	20 °C, 9 h	$(C_2H_5)_2N$-	**25**	78
	20 °C, 1 h		**26**	74 (45)[a]
	20 °C, 2 h		**27**	78 (36)[a]
	20 °C, 28 h		**28**	60 (43)[a]
 **21**	20 °C, 12 h		**29**	85 (60)[a,b]
$CH_3NH\text{-}Si(CH_3)_3$ **22**	20 °C, 1 h	CH_3NH-	**30**	70
$H_2N\text{-}Si(C_2H_5)_3$ **23**	50 °C, 18 h	H_2N-	**31**	45[c]
$Me_3Si\text{-}NH\text{-}SiMe_3$	50 °C, 22 h	H_2N-	**31**	40[c]

[a] yield of amino acid from direct reaction of parent amine with **7**; amide is also generated. [b] The authentic sample of the corresponding amide **32** was made (see expt). [c] Polymerized material was also obtained.

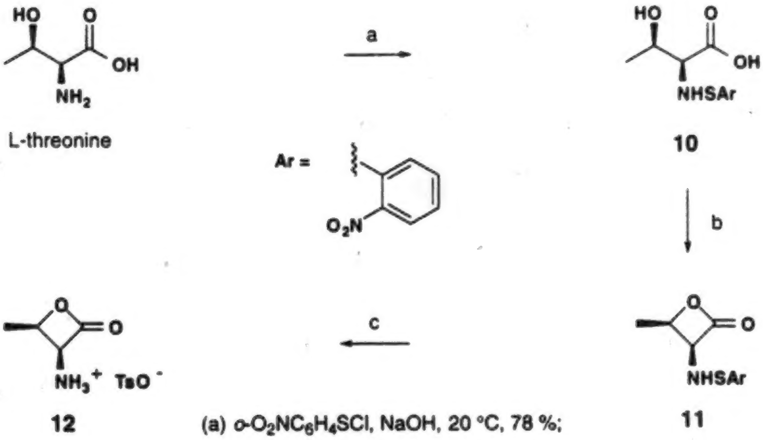


Scheme 5.

II Studies on *N*-(*o*-Nitrophenyl)sulfenyl-L-Threonine β-Lactone 11.

1. Ring Opening With Aluminum-Amine Complexes.

L-Threonine β-lactone **11** was prepared according to the method developed by Vederas.[84] Thus, L-threonine was protected with the (2-nitrophenyl)sulfenyl group to form acid **10**, which was cyclized *via* carbonyl group activation with 4-bromophenylsulfonyl chloride in pyridine, to give L-threonine β-lactone **11**. Treatment of **11** with *p*-thiocresol and TsOH generated the stable tosylate salt **12** (Scheme 6).


HO
O
OH
NH$_2$
L-threonine
a
NHSAr
10
Ar =
O$_2$N
b
c
NH$_3^+$ TsO$^-$
12
11

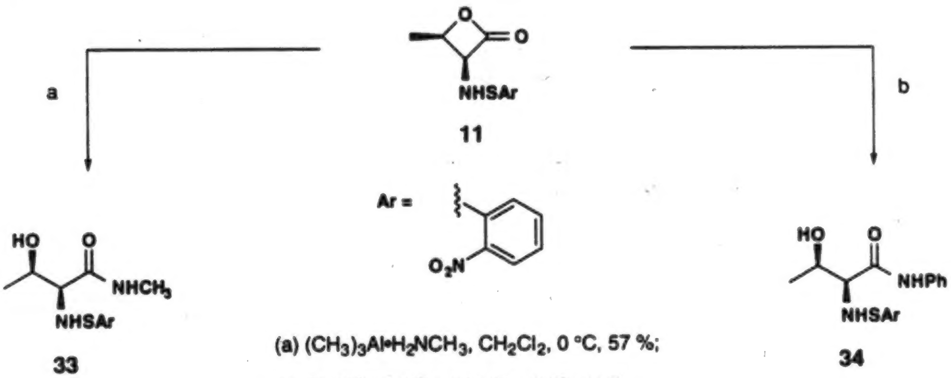
(a) o-$O_2NC_6H_4SCl$, NaOH, 20 °C, 78 %;

(b) p-$BrC_6H_4SO_2Cl$, pyr, -40 to 0 °C, 55 %;

(c) p-$CH_3C_6H_4SH$, TsOH , CH_2Cl_2, 76 %.

Scheme 6.

L-Threonine β-lactone **11** behaved similarly to L-serine β-lactone **7** in its reactions with aluminum•amine reagents. The ring opening proceeded by acyl-oxygen cleavage to produce threoninamides (Scheme 7).


a
b
NHSAr
11
Ar =
O$_2$N
HO
O
NHCH$_3$
33
NHPh
34

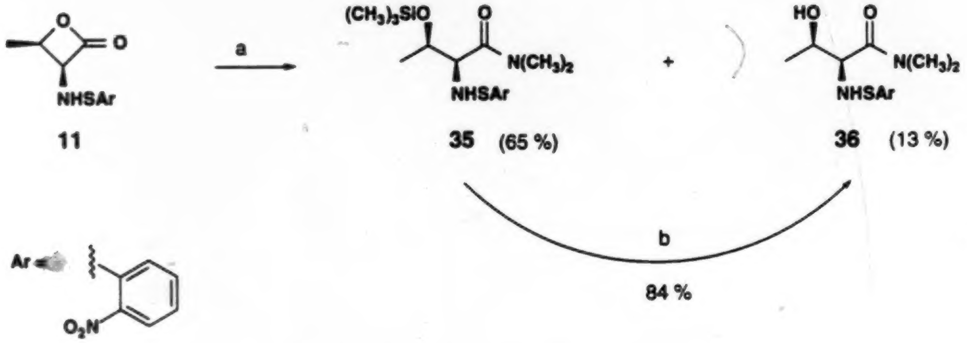
(a) $(CH_3)_3Al•H_2NCH_3$, CH_2Cl_2, 0 °C, 57 %;

(b) $Et_2AlCl•H_2NPh$, CH_2Cl_2, 0 °C, 40 %.

Scheme 7.

2. Ring Opening with *N*-Silylamine Reagents.

Preliminary experiments showed that the reaction of *N*-silylamines with threonine β-lactone **11** proceeded by attack at the carbonyl to form amides. For example, attempts to open β-lactone **11** with *N*-silylamine **19** failed to produce any of the β-substituted product, with amides **35** and **36** being produced instead (Scheme 8). This was in contrast to the facile ring opening at the methylene carbon of the serine β-lactones **7**. It was However in accord with the finding[76] that the ring opening of threonine β-lactones, such as **11**, with nitrogen nucleophiles like pyrazole and benzylamine occured primarily (if not exclusively) at the carbonyl to form amides.



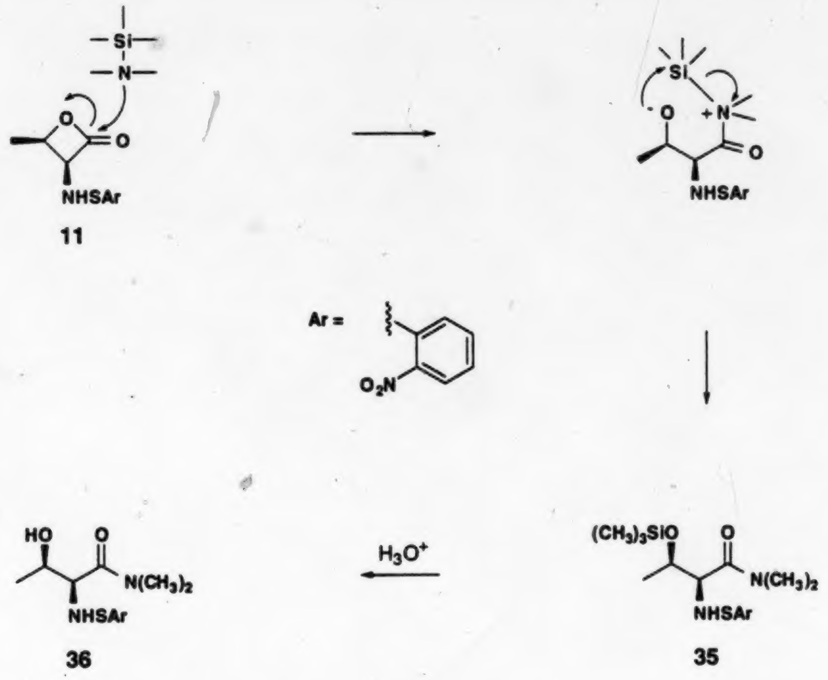
(a) $(CH_3)_2N\text{-}Si(CH_3)_3$, CH_3CN, 50 °C, 48 h;

(b) TBAF, THF, 20 °C.

Scheme 8.

Apparently, the additional steric effect due to the β-methyl group and/or electron-withdrawing effect of the α-nitrogen substituent on the β-lactone ring (e.g. **11**) suffices to alter the course of reaction from that observed with the less-substituted β-propiolactone[106c] and serine β-lactones (e.g. **7**).[71-74]

The silyl ether derivative **35** can be isolated and is likely to be a precursor of **36**. As shown in Scheme 8, treatment of **35** with TBAF generated **36** in 84 % yield. It seems that during the work up of the reaction, some hydrolysis of **35** to the alcohol **36** occurred. A proposed mechanism for the formation of **36** is shown in Scheme 9.


Si
N
O
O
NHSAr
11
Si
O
+N
O
NHSAr
Ar =
O_2N
HO
O
N(CH₃)₂
NHSAr
36
H₃O⁺
(CH₃)₃SiO
O
N(CH₃)₂
NHSAr
35

Scheme 9.

Although at present the unexpected tendency of threonine β-lactones to undergo carbonyl attack limits their utility for the synthesis of new amino acids, the correct choice of their *N*-protecting group may allow the synthesis of such compounds.

CHAPTER 2 Inhibition of Protein Prenyl Transferases

INTRODUCTION

1 Background.

Farnesyl pyrophosphate (FPP), a product of the mevalonic acid biosynthetic pathway,[113] is regarded as the last common substrate for the so-called branch-point enzymes, i.e. the enzymes catalyzing the first committed steps in the biosynthesis of isoprenylated proteins, cholesterol, ubiquinone, dolichol, and heme a (Figure 14).[114]

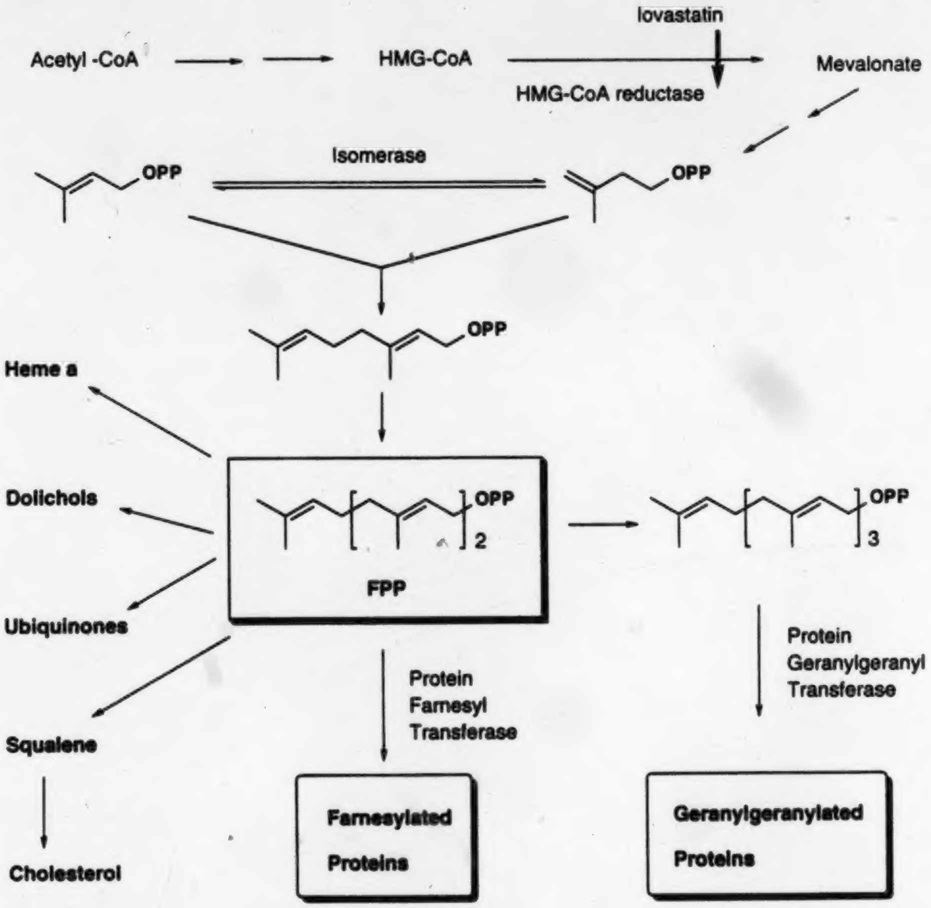

lovastatin
Acetyl -CoA
HMG-CoA
HMG-CoA reductase
Mevalonate
Isomerase
OPP
OPP
OPP
Heme a
Dolichols
Ubiquinones
Squalene
Cholesterol
OPP
2
FPP
OPP
3
Protein Farnesyl Transferase
Protein Geranylgeranyl Transferase
Farnesylated Proteins
Geranylgeranylated Proteins

Figure 14. Biosynthesis and metabolism of isoprenoid derivatives.

Protein isoprenylation[115] involves the addition of either the C15 isoprenoid farnesyl or the C20 isoprenoid geranylgeranyl to a cysteine residue at, or near the C-termini of proteins. Among the most studied family of isoprenylated proteins are Ras proteins, the products of *ras* genes,[116] which are functionalized for biological activity by farnesylation followed by other processing steps (Figure 15). Ras proteins are members of the low molecular weight GTP-binding proteins[117] that bind to guanine nucleotides GTP and GDP and possess intrinsic GTPase activity. They are active in the GTP-bound conformation and inactive in the GDP-bound state.[118]

Farnesylation[119] (Step 1, Figure 15) has been identified as the critical post-translational modification that is necessary for the translocation, and subsequent cell-transforming activity of Ras proteins.[120] Mutated forms of *ras* genes are found in 25% of all human tumors and the rate of incidence is even higher (> 50%) in colon and pancreatic cancers.[121] Mutations which abolish the intrinsic activity of Ras proteins result in their inability to hydrolyze GTP and they become locked in the biologically active GTP-bound state, thereby triggering a continuous growth signal which leads to malignant transformation.[122] Thus, inhibition of the Ras farnesylation reaction would suppress *ras*-mediated tumor growth.

Several strategies have been employed to inhibit Ras farnesylation. These include inhibition of isoprenoid biosynthesis and inhibition of the enzyme which catalyzes the farnesylation reaction, protein farnesyltransferase (PFTase). Although inhibitors of the rate limiting enzyme in isoprenoid biosynthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (see Figure 14), such as lovastatin,[123] block farnesylation of Ras, they also deplete the cell of mevalonate and consequently farnesyl pyrophosphate which is essential for other biosynthetic pathways. Furthermore, relatively high concentrations of lovastatin are required to inhibit Ras farnesylation in cultured cells,[124] giving rise to cell toxicity. Therefore, specific inhibition of protein farnesyltransferase (PFTase) is a more attractive approach.

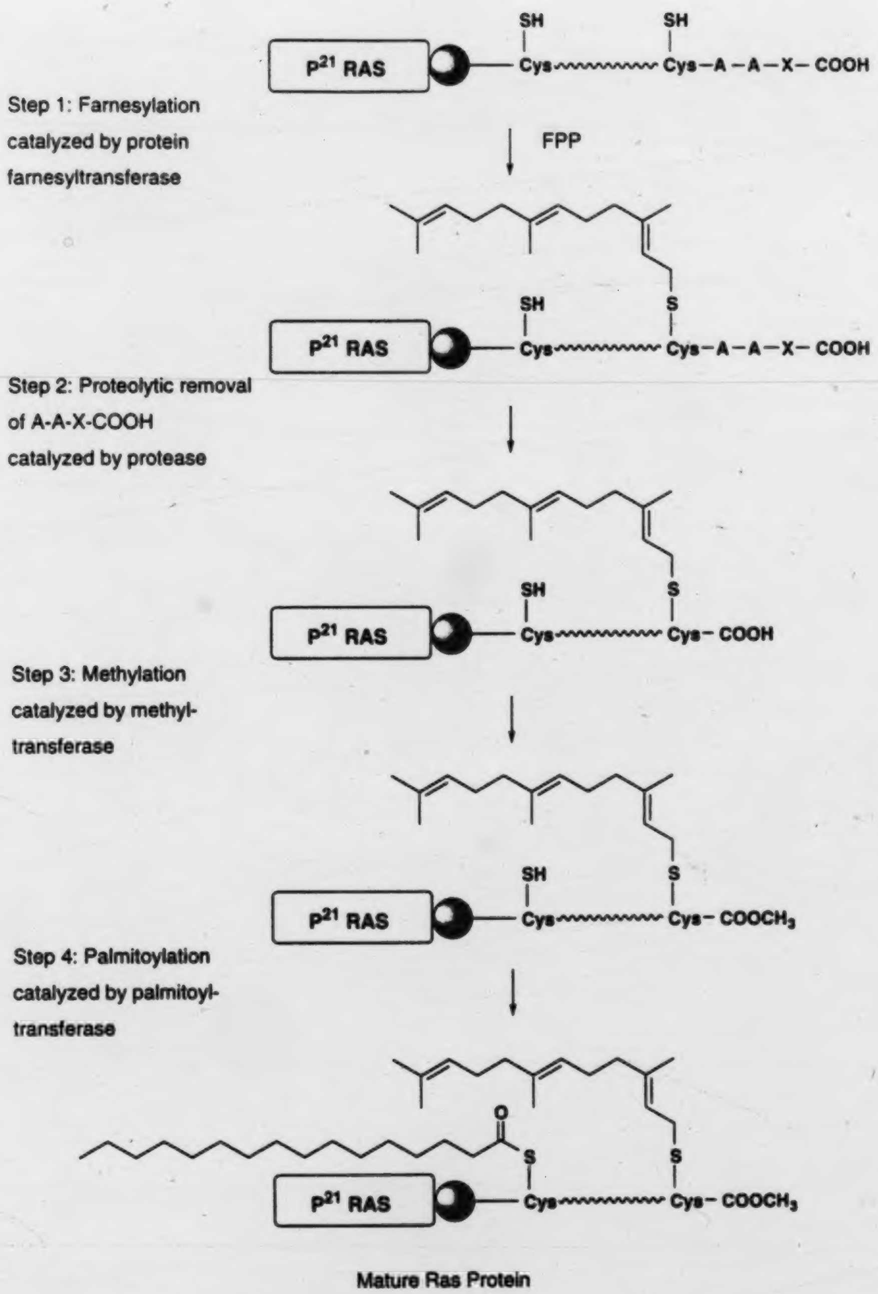

P21 RAS
SH
SH
Cys
Cys—A—A—X—COOH
Step 1: Farnesylation catalyzed by protein farnesyltransferase
FPP
SH
S
Cys—A—A—X—COOH
Step 2: Proteolytic removal of A-A-X-COOH catalyzed by protease
Cys—COOH
Step 3: Methylation catalyzed by methyl-transferase
Cys—COOCH3
Step 4: Palmitoylation catalyzed by palmitoyl-transferase
O
S
Mature Ras Protein

Figure 15. Post-translational modification steps in the processing and formation of functionalized Ras proteins.

2 Protein Prenyltransferases:

Three kinds of prenyltransferases have been identified: protein farnesyltransferase (PFTase),[119a] protein geranylgeranyltransferase type I (PGGTase-I),[125] and protein geranylgeranyltransferase type II (PGGTase-II).[126] These enzymes are found in mammalian and yeast cells and recently[127] one of them (PFTase) has been identified in spinach. Cellular proteins serving as substrates for PFTase and PGGTase-I share a characteristic *C*-terminal sequence referred to here as CAAX, where C is cysteine, A can be any amino acid (usually aliphatic), and X is a restricted amino acid whose nature determines the type of isoprenoid modification of the cysteine residue.[120,128] PGGTase-II recognizes substrates terminating in XXCC, XCXC and CCXX in which the position of the cysteine residue is not as restricted as in the case of PFTase and PGGTase-I.[126]

2.1 Protein Farnesyltransferase (PFTase).

Protein farnesyltransferase is the most studied enzyme of the three prenyltransferases identified to date, partly because it is the only one which has been purified to homogeneity. The enzyme has been isolated from rat brain cytosol and shown to be a heterodimeric protein composed of α- and β-subunits with molecular masses of 48 kDa and 46 kDa, respectively.[129] Both subunits are required for catalytic activity and both have been cloned.[130] The yeast[131] and human[132] forms of PFTase have also been cloned and sequenced. PFTase recognizes proteins that terminate in the sequence CAAX where C is cysteine, A is an aliphatic amino acid and X is methionine, serine, glutamine, or cysteine.[133] Protein substrates of PFTase include the Ras proteins, the nuclear lamins, and the α-and β-subunits of skeletal muscle phosphorylase kinase.[121b,134] The other substrate involved in the farnesylation of these proteins by PFTase is farnesyl pyrophosphate (FPP).

PFTase catalyzes the transfer of a farnesyl group from FPP to a cysteine residue that is fourth in line from the carboxyl terminus of $p^{21 ras}$ proteins (Figure 16). The β-subunit binds the acceptor protein, in a zinc-dependent process, while the α-subunit has been postulated to play a role in the binding of FPP, an event which requires Mg.[135]

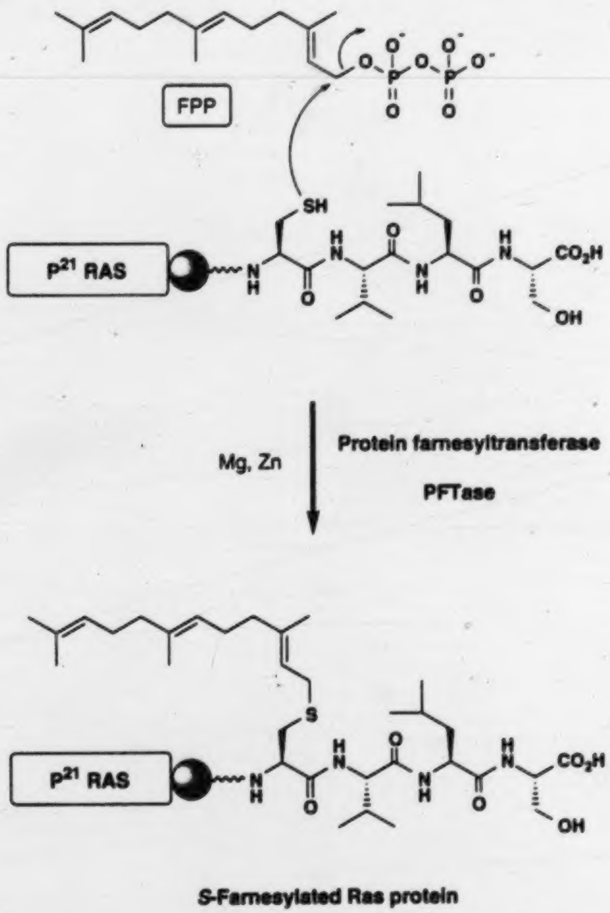

FPP
SH
P21 RAS
CO2H
OH
Mg, Zn
Protein farnesyltransferase
PFTase
S
P21 RAS
CO2H
OH
S-Farnesylated Ras protein

Figure 16. PFTase-catalyzed farnesylation of $p^{21 ras}$

The reaction mechanism of the yeast[136] PFTase and human[137] PFTase has recently been studied.

2.2 Inhibition of PFTase.

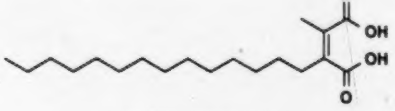
Inhibitors of PFTase have been identified both through targeted screens and rational design based on the structures of the two substrates of the reaction.

2.2.1 Inhibitors From Natural Sources.

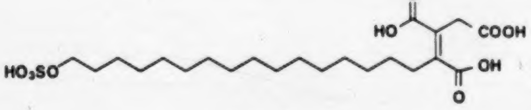
The natural inhibitors reported to date can be divided into three main classes: (a) inhibitors that are competitive with the natural substrate farnesyl pyrophosphate (FPP), including CP-225,917,[138] oreganic acid,[140] zaragozic acids,[140] actinoplanic acids,[141] chaetomellic acids,[142] and manumycin analogues;[143] (b) inhibitors which are competitive with the Ras peptide, such as pepticinnamins[144] and (c) inhibitors which are either not competitive with either of the PFTase substrates or whose mechanism of inhibition is unknown. The last class of inhibitors includes andrastins,[145] cylindrols,[146] SCH 58450,[147] preussomerins,[148] fusidienol,[149] gliotoxin,[150] and 10'-desmethoxystreptonigrin.[151] Figure 17 shows the structures of some representatives of the three classes.

2.2.2 Rationally Designed Inhibitors.

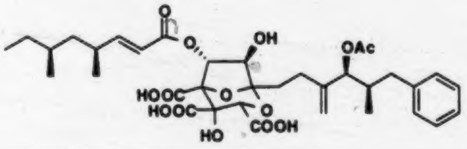
Since PFTase catalyzes a bi-substrate reaction (Figure 16), the design of inhibitors can be based on either one or both of the two natural substrates. This section presents recent developments in the design of inhibitors based on: a) the Ras CAAX tetrapeptide motif b) farnesyl pyrophosphate (FPP), and c) a combination of the two substrates.



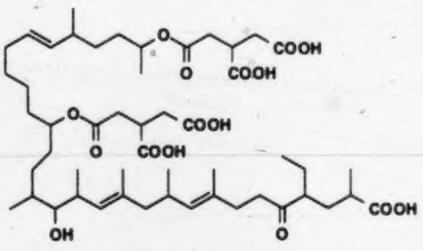
Chaetomellic acid A



Oreganic acid

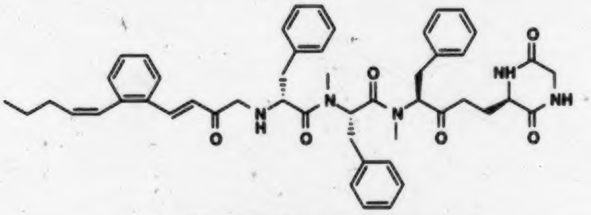


Zaragozic acid A



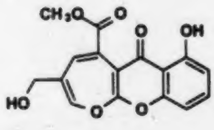
Actinoplanic acid B

Natural inhibitors which are competitive with FPP

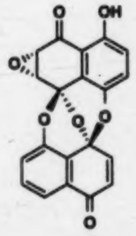


Pepticinnamins

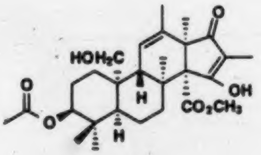
Natural inhibitors which are competitive with Ras peptide.



Fusidienol



Preussomerin G



Andrastin B

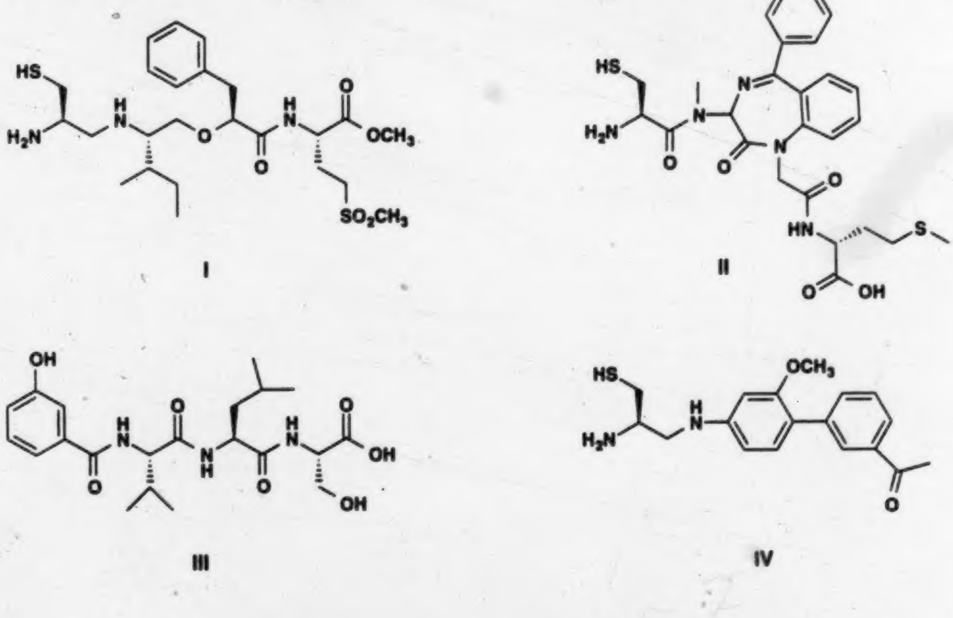
Natural inhibitors of PFTase which are not competitive with either substrate

Figure 17. Different types of natural inhibitors of protein farnesyltransferase.

a) Inhibitors based on Ras CAAX tetrapeptide mimics.

Tetrapeptides containing a CAAX sequence were the first class of inhibitors of the enzyme to be studied.[119a] Recent findings show that CAAX peptidomimetics[152-154] which are potent and selective inhibitors of PFTase are potential anti-cancer agents. They have been shown to cause inhibition of *ras*-dependent tumor growth in nude mice[155-157] and to selectively block oncogenic H-Ras signaling and the growth of murine[158] and human[159] tumors in animal models, with minimal toxic effects.

Figure 18 shows different types of CAAX-based inhibitors. Stabilization of the peptide bonds by reduction (compound **I**)[152e,156] or by *N*-methylation (compound **II**)[152g] yielded analogues which were resistant to degradation by cellular proteases. Compound **III** represents a unique family of inhibitors[152f] in which the phenolic hydroxyl serves as a suitable replacement for the sulfhydryl group. Compound **IV** represents a class of non-peptide[153] Ras CAAX mimetics which are potent inhibitors of PFTase.



I **II**

III **IV**

Figure 18. Synthetic CAAX-based inhibitors of PFTase.

b) Inhibitors based on farnesyl pyrophosphate (FPP).

Inhibitors of PFTase which compete with FPP have been discussed in a recent review.[160] Since then numerous reports have appeared describing the design of potent inhibitors of PFTase based on mimicking FPP. In these designs the labile polyanionic pyrophosphate group has been replaced by stable synthetic surrogates.[161] To gain information about the interactions between PFTase and FPP and the mechanism of prenyl transfer, photoreactive inhibitor analogues[162] and substrate analogues,[135,163] originally prepared as mechanism-based inhibitors, have been prepared. In designing substrate-based inhibitors for a certain enzyme, the issue of specific inhibition is important. For example, selective PFTase inhibitors are likely to elicit fewer cytotoxic effects than a non-selective inhibitor of PFTase, PGGTase and squalene synthase.[164] Some examples of these FPP-mimic inhibitors of PFTase, along with FPP for comparison, are shown in Figure 19.

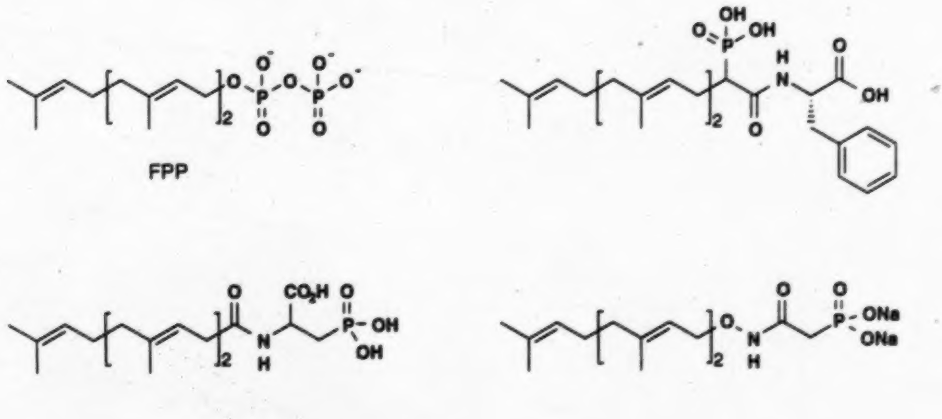
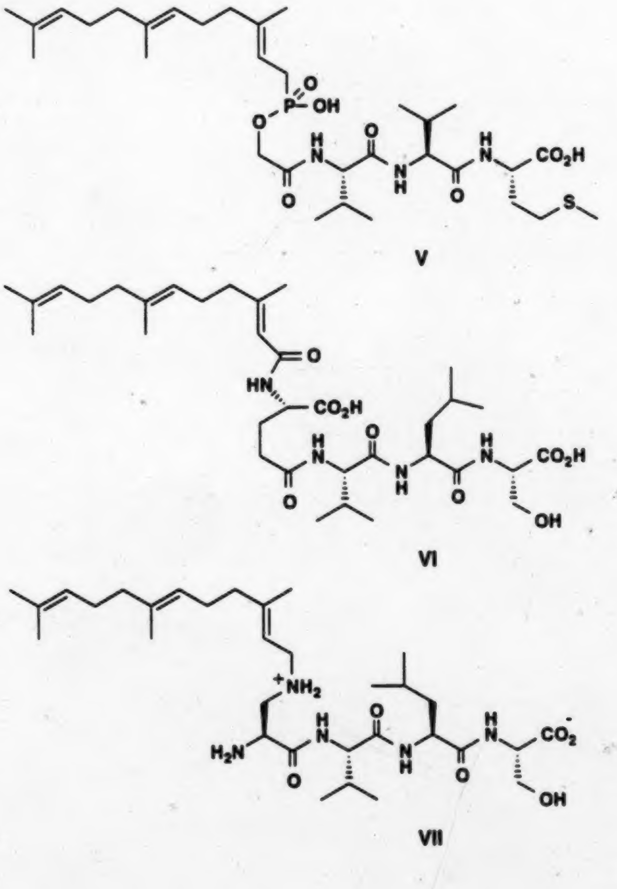


Figure 19. FPP-based inhibitors of PFTase.

c) Bi-substrate analogue inhibitors.

The inhibitor design strategy of this type of analogues involves hybridization of the two substrates into a single chemically and biologically stable entity. Figure 20 shows three examples (**V-VII**) of potent non-sulfhydryl bi-substrate analogue inhibitors of PFTase. In these compounds, the farnesyl group has been retained to preserve putative hydrophobic interactions, and the Ras *C*-terminal tripeptide was chosen as the peptide substrate component. The phosphonic acid[165] in compound **V** and the carboxylic acid[166] in **VI** serve as mimics of the sulfhydryl group. Unlike the tetrapeptide-based inhibitors, a free sulfhydryl group was not a requirement for the activity of these bi-substrate inhibitors. The bi-substrate inhibitor **VII** was designed as a transition state analogue for PFTase.[167]



V

VI

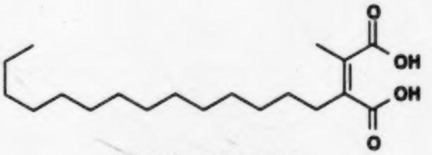
VII

Figure 20. Bi-substrate-based inhibitors of PFTase.

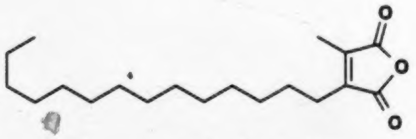
Based on the phenomenon of "feedback inhibition", which is common in enzyme-mediated reaction cascades, a recent report[168] showed that a lipohexapeptide representing the completely functionalized, i.e. farnesylated and palmitoylated (see Figure 15), *C*-terminus of the human *N*-Ras protein is a weak inhibitor of PFTase.

3 Synthesis of Chaetomellic Acid A.

Chaetomellic acid A is one of several polycarboxylic acid-containing natural products (see Figure 17) identified as potent inhibitors of Ras PFTase. Chaetomellic acid A, isolated from *Chaetomella acutiseta* as its anhydride, is a nanomolar (55 nM) specific competitive inhibitor of PFTase with respect to FPP.[119c,142]



Chaetomellic acid A



Chaetomellic acid A anhydride

To date, six syntheses of chaetomellic acid A anhydride have been reported, including the one developed[169] in the Vederas group which will be discussed in part 1 of the following chapter (*vide infra*).

The first reported synthesis of chaetomellic acid A anhydride involved the non-stereospecific aldol condensation of methyl palmitate with methyl pyruvate to provide the anhydride in 18% overall yield.[170] The second synthesis relied on a doubly chemoselective radical cross coupling of myristyl cobaoxime with citraconic anhydride and diphenyl disulfide to give the anhydride in 64% overall yield.[171] The third synthesis of chaetomellic acid A anhydride, which appeared in the literature after our approach had been accepted for publication, involved a novel succinate to maleate oxidation and was achieved in 83% overall yield.[172] The fourth synthesis used the condensation of tetradecylimidazopyridinium bromide and maleic anhydride to give chaetomellic acid A anhydride in 62% overall yield.[173] The most recent synthesis involved a Wittig reaction between a citraconimide derivative and tetradecanal to provide chaetomellic acid A anhydride in 89% overall yield.[174]

In the present work, we describe:

(1) The development of a convenient two-step, stereospecific preparation of chaetomellic acid A using a tandem vicinal difunctionalization of dimethyl acetylenedicarboxylate (DMAD).

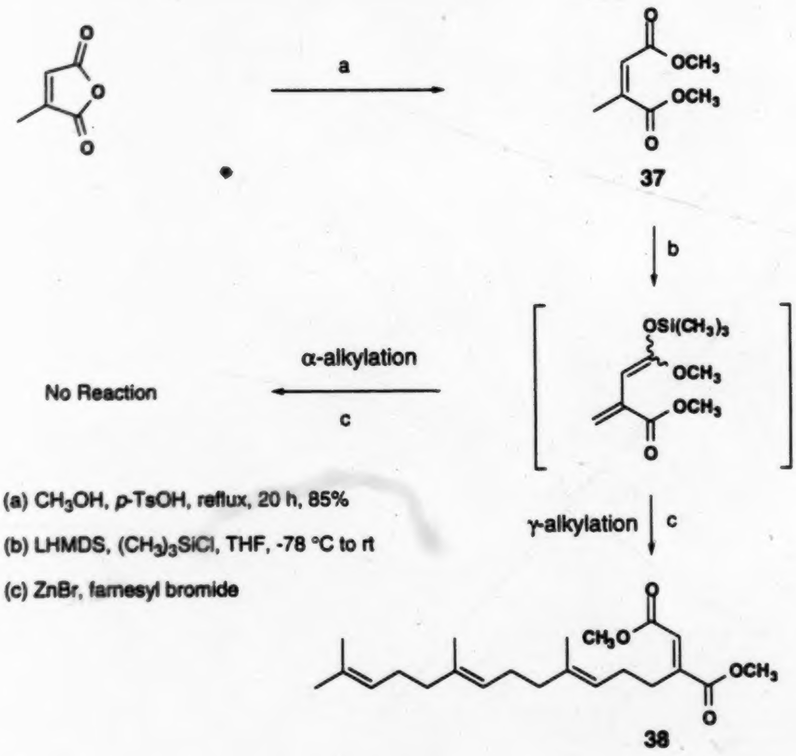
(2) The use of this methodology in the preparation of other specific inhibitors of both protein farnesyltransferase (PFTase) and protein geranylgeranyltransferase (PGGTase).

RESULTS AND DISCUSSION

I Inhibition of PFTase and PGGTase.

1 Synthesis of Chaetomellic Acid A and Derivatives.

Intially experiments focused on an alkylation approach involving carbon-carbon bond formation at the α- and/or γ-position of a dienolate (Scheme 10).[175] Despite the different reaction conditions employed, including the use of reactive electrophiles for the alkylation step, the reaction products obtained were complex mixtures and low yields of alkylated products were recovered. In the most successful case, where farnesyl bromide was used for the alkylation of maleate ester **37**, the γ-alkylation product **38** was isolated in only 2% yield. This approach was therefore abandoned.



(a) CH_3OH, *p*-TsOH, reflux, 20 h, 85%

(b) LHMDS, $(CH_3)_3SiCl$, THF, -78 °C to rt

(c) ZnBr, farnesyl bromide

Scheme 10.

Next, a conjugate addition/enolate trapping approach was examined. The synthetic methodology which is based on tandem vicinal difunctionalization of dimethyl acetylenedicarboxylate (DMAD) is shown in Figure 21.[176] This strategy, if successful, would prove to be a versatile method for the construction of the entire skeleton of chaetomellic acid in a single step. Furthermore, it would allow for rapid access to other analogues.

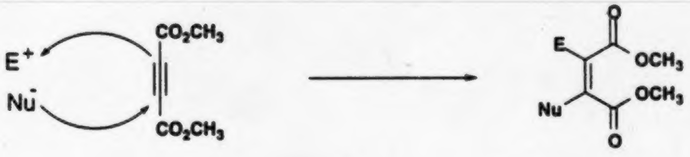
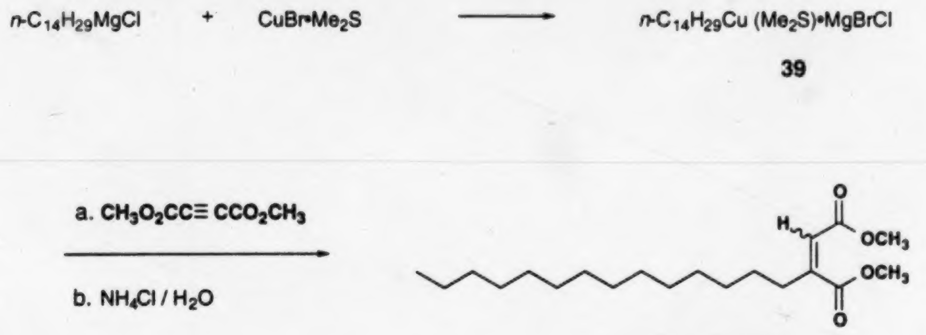


Figure 21. Tandem vicinal difunctionalization of DMAD.

The conjugate addition of organocuprates (obtained by treatment of Grignard and organolithium reagents with copper (I) salts) to alkynes is a widely used synthetic methodology.[177,178] A closer investigation of the literature revealed that α-functionalization (*via* enolate trapping) of acetylenes bearing electron withdrawing substituents,[179] such as acetylene dicarboxylates,[180] has limitations imposed by the instability of copper intermediates at the higher temperatures necessary for certain α-functionalizations. For example, methyl propynoate is reported to undergo conjugate addition, but attempts to alkylate the vinyl copper intermediate were often unsuccessful.[181]

Initial experiments using an aqueous quench showed that solvent, temperature, and reaction time influence the stereochemical outcome of the conjugate addition of Grignard-derived tetradecyl organocuprate **39** (Scheme 11) to dimethyl acetylenedicarboxylate (Table 3). Reaction in THF at -78 °C for a short period (45 min) gave exclusively *cis*-addition of the tetradecyl moiety and proton to generate the *Z*-diester

40a. However, prolonged reaction time (3 h), higher temperature (- 40 °C), or use of ether as a solvent[182] all led to a rapid deterioration in the stereospecificity. Addition of dimethyl sulfide as a cosolvent to ether (1:2)[183] allowed some recovery of the ratio.


n-$C_{14}H_{29}MgCl$ + $CuBr{\bullet}Me_2S$ → n-$C_{14}H_{29}Cu\ (Me_2S){\bullet}MgBrCl$
39
a. $CH_3O_2CC{\equiv}CCO_2CH_3$
b. NH_4Cl / H_2O
H
O
OCH₃
OCH₃
O

40a: *Z*-isomer

40b: *E*-isomer

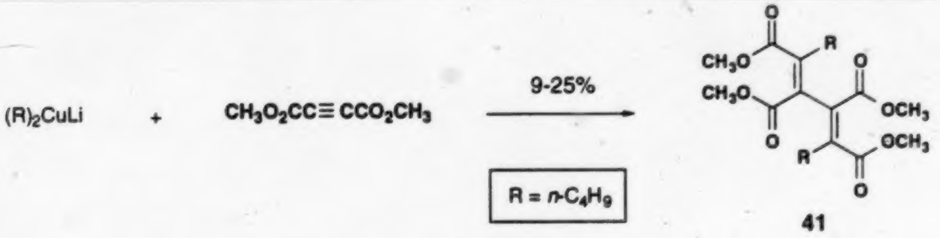
Scheme 11. See Table 3.

Table 3. Conjugate Addition of **39** to Dimethyl Acetylenedicarboxylate (DMAD).

Conditions				Products	
Entry	Solvent	T (°C)	Time[a] (h)	*Z*:*E* Ratio (**40a**:**40b**)[b]	Yield[c] (%)
1	THF	- 78	0.45	100:0	85
2	THF	- 78	3	95:5	83
3	THF	- 40	3	88:12	75
4	ether	- 78	1	78:22	78
5	ether	- 40	1	60:40	78
6	ether-Me_2S	- 40	1	85:15	60

[a] Time between addition of DMAD and quenching of reaction. [b] Determined by ^1H NMR spectroscopy. [c] Isolated yield.

Furthermore, use of other organocopper-derived reagents gave less satisfactory results. For example, attempted alkylation of DMAD by the dialkylcopper reagent $(n\text{-Bu})_2CuLi$ (prepared from 2 *n*-BuLi and CuI) resulted in a complex mixture of products, where the only isolable compound was diene **41** (9% yield). Alkylation by the same reagent, $(n\text{-Bu})_2CuLi$, prepared differently from 2 *n*-BuLi and $CuBr{\bullet}Me_2S$ also gave diene **41** in 25% yield (Scheme 12).



Scheme 12.

The loss of stereospecificity at higher temperatures is probably due to equilibration of the "enolate" adducts (vinyl copper adducts),[184] resulting from the conjugate addition, which is very slow at -78 °C but becomes rapid above -40 °C, giving mixtures of the *Z* -and *E*-isomers (Figure 22).

The stereochemical assignment of **40a** and **40b** was initially based on the chemical shifts of the vinylic hydrogens which should be more downfield in the case of the *E*-isomer, based on a comparison of the chemical shifts of known compounds. Indeed the ^1H NMR spectrum showed that in the *Z*-isomer **40a** the vinylic hydrogen resonates at 5.81 ppm where in the *E*-isomer **40b** it resonates at 6.72 ppm. This is due to the deshielding effect of the ester group that is in the β-position relative to the vinylic hydrogen.

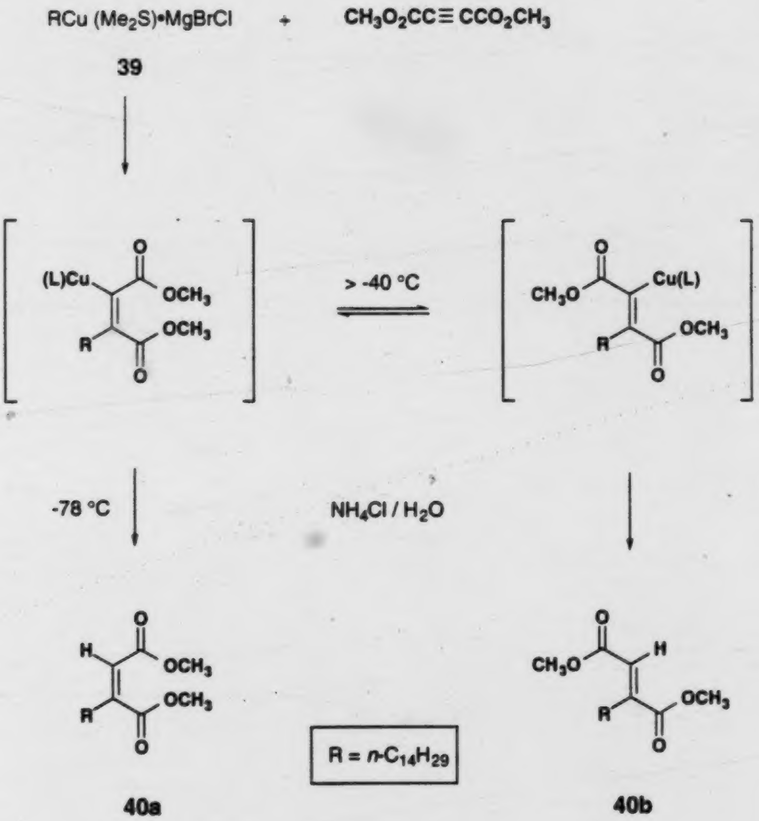

RCu (Me2S)•MgBrCl
+
CH3O2CC≡CCO2CH3
39
(L)Cu
OCH3
OCH3
R
O
O
> -40 °C
CH3O
Cu(L)
OCH3
-78 °C
NH4Cl / H2O
H
R = n-C14H29
40a
40b

Figure 22.

Further evidence for the olefin stereochemical assignment was obtained from nOe studies (Figure 23).[185] In the case of **40a,** irradiation of the vinylic hydrogen showed a 2 % enhancement of the allylic hydrogens' signal, while irradiation of the allylic hydrogens resulted in an enhancement of the vinylic hydrogen by 16 %. The strong nOe indicates that these protons are close in space, which is in agreement with the (*Z*)-configuration of the double bond. No such nOe was observed in the case of the *E*-isomer **40b**.

(*Z*)-configuration of the double bond. No such nOe was observed in the case of the *E*-isomer **40b**.

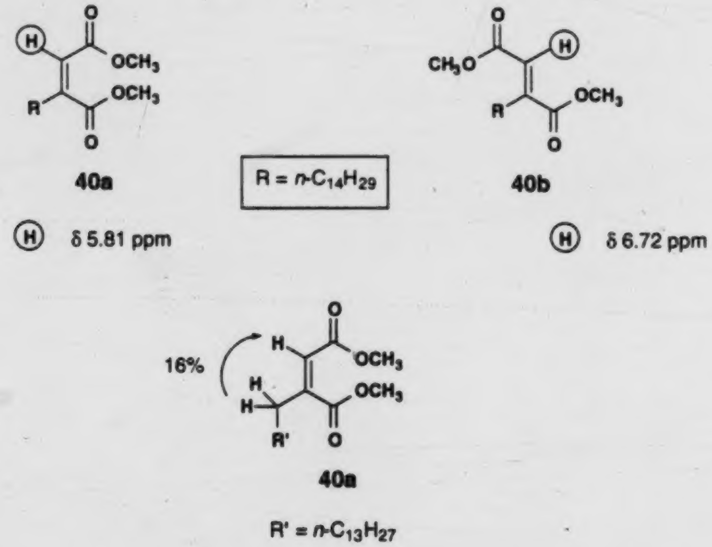
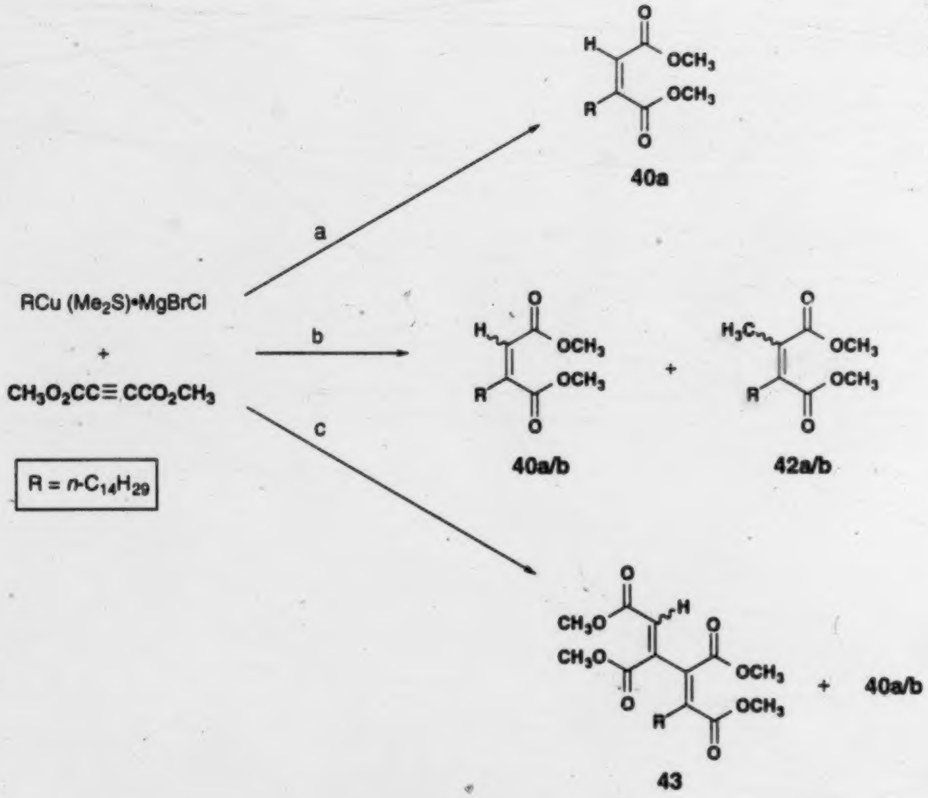


Figure 23.

Having established the right conditions for the conjugate addition reaction to afford exclusively the desired *Z*-isomer, we turned our attention to the enolate trapping part of the reaction (see Figure 21). For the synthesis of chaetomellic acid A, the electrophile for the enolate trapping had to be a methylating agent. Attempts to generate tetra-substituted olefins by capture of the copper enolate in THF at -78 °C with reactive methylating agents such as MeI or $(Me)_3O^+BF_4^-$ failed and gave only the *Z*-diester **40a** upon workup. Performing the reaction at temperatures higher than -40 °C provided the desired methylated product **42**, but only in low yield and as a mixture of isomers. The major product was **40** obtained again as a mixture of *Z*- and *E*-isomers. When the reaction was conducted in ether and MeOTf was used as the methylating agent,[186] the major



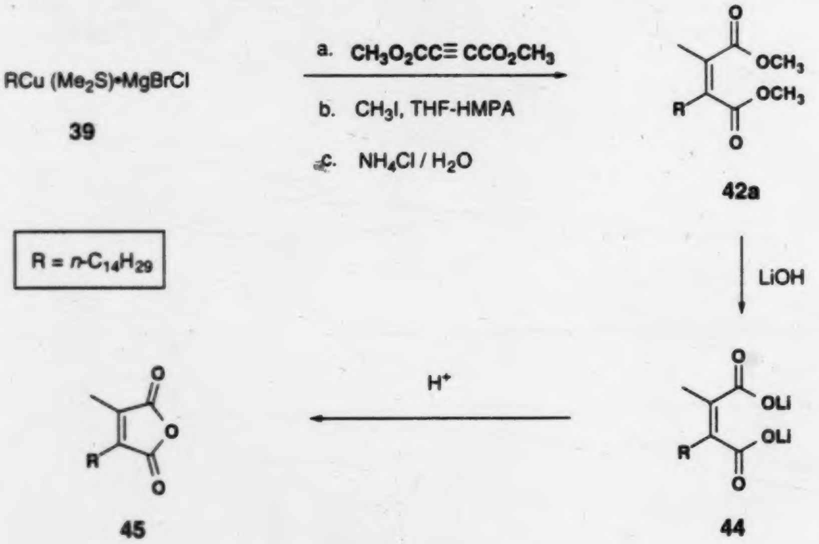
(a) CH_3I or $(CH_3)_3O^+ {}^-BF_4$, THF, -78 °C, then H^+;

(b) CH_3I or $(CH_3)_3O^+ {}^-BF_4$, THF, -78 °C to rt, then H^+;

(c) CH_3OTf, ether, -78 °C, then H^+.

Scheme 13.

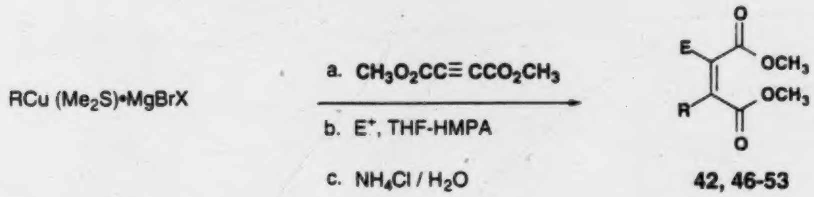
At this stage it became clear that in order to have an efficient, stereoselective enolate trapping reaction, the conjugate addition adduct had be stabilized at higher temperatures. We found that complexation with HMPA in THF was highly effective[187] at stabilizing the enolate adduct resulting from conjugate addition and greatly retards its equilibration or thermal decomposition even at 20 °C. Thus, Michael addition of the organocopper reagent **39** to DMAD in the presence of HMPA, followed by capture of the resulting enolate with methyl iodide, generated chaetomellic acid A methyl ester **42a** (Scheme 14) in 78 % yield. Careful hydrolysis with lithium hydroxide afforded chaetomellic acid A di-lithium salt **44** in quantitative yield. Salt **44** cyclizes rapidly to the corresponding anhydride **45** in the presence of acid. This is the simplest and most efficient approach to this compound reported thus far.



Scheme 14.

A variety of other electrophiles also capture such copper enolates effectively. These include allylic halides (farnesyl, geranylgeranyl or geranyl bromide), acylating agents (tetradecanoyl chloride), *N*-bromosuccinimide, and trimethyltin chloride (Table 4). Reaction yields depend on the quality of the cuprous bromide-dimethylsulfide complex, $CuBr{\bullet}Me_2S$. Use of the reagent that is freshly prepared[188] or freshly obtained in high purity from commercial sources is essential for satisfactory results. Aged bottles of $CuBr{\bullet}Me_2S$ (Aldrich) gave inferior yields of product. Recrystallization[189] of the salt prior to use increased yields considerably.

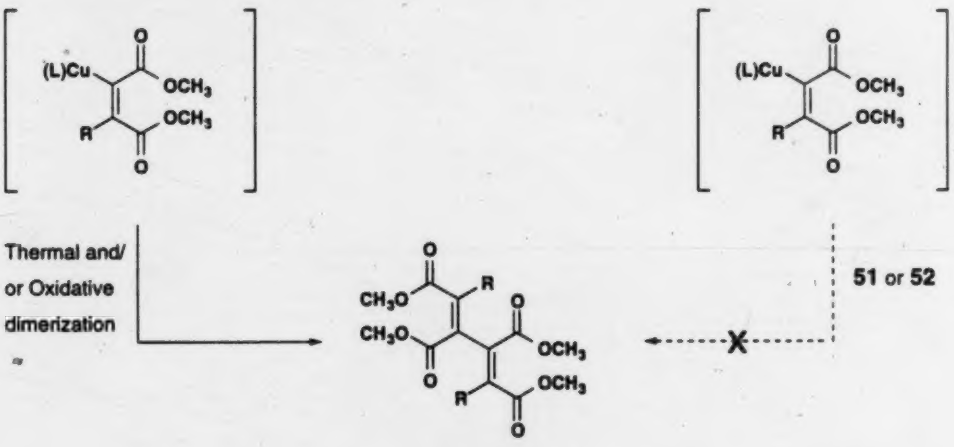
Table 4. Tandem addition to dimethyl acetylenedicarboxylate (DMAD).



Entry	R	E+ X-	Product	Yield (%)[a]
1	n-$C_{14}H_{29}$	CH_3I	**42a**	78
2	n-$C_{12}H_{25}$	CH_3I	**46**	77
3	CH_3	farnesyl-Br	**47**	80
4	CH_3	geranylgeranyl-Br	**48a**	81
5	CH_3	geranyl-Br	**49a**	85
5	CH_3	$C_{13}H_{27}COCl$	**50**	83
6	n-$C_{14}H_{29}$	NBS[b]	**51**	67
7	CH_3	NBS[b]	**52**	75
8	CH_3	$(CH_3)_3SnCl$	**53**	49

[a] Isolated yields. [b] *N*-Bromosuccinimide; E^+= Br.

During the preparation of **51** and **52**, dienes **54** and **55** (Figure 24) were formed, respectively, as by-products (< 5%), presumably because of dimerization of the vinyl copper intermediate (R = CH_3 or n-$C_{14}H_{29}$, Figure 22). Diene **55** was also isolated during the preparation of **53**. Symmetrical dienes[190] are known to be generated *via* thermal[191] or oxidative[192] dimerization of vinylcopper reagents. The influence of HMPA on the yields of compounds **51-53** was very pronounced. In the absence of HMPA the amounts of dienes **54** and **55** were appreciable (10-25 %) and the reaction was noticeably slower. The amounts of the dienes formed also increased when the reaction temperature was raised above -40 °C. Apparently, HMPA is exerting a pronounced stabilizing effect on the vinylcopper intermediate and suppressing any oxidative dimerization by NBS[193]. HMPA may also be breaking up the cluster structure[194] in which the vinyl copper intermediates are most likely present.

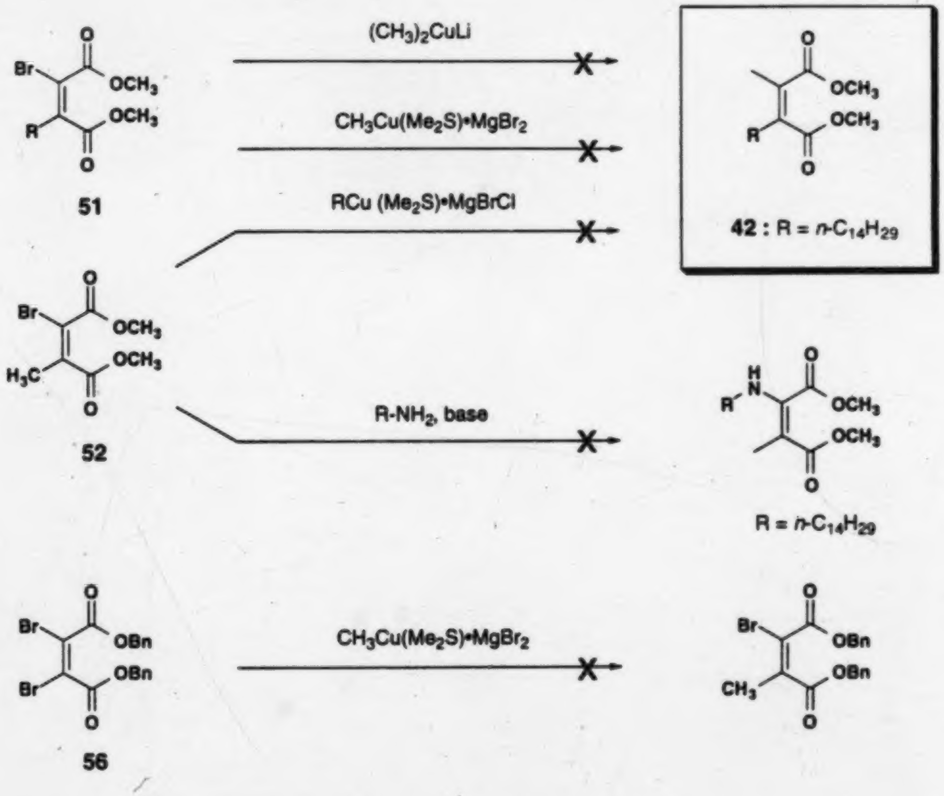


54 : R = n-$C_{14}H_{29}$

55 : R = CH_3

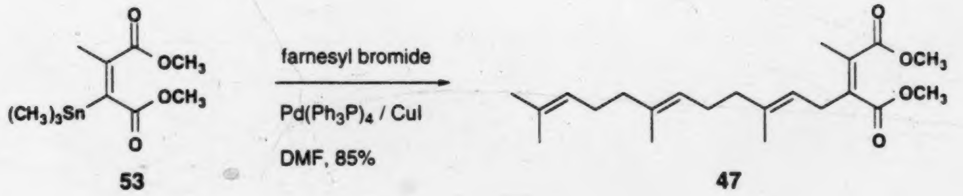
Figure 24.

Vinyl bromides **51** and **52** were intially designed to be used in an addition-elimination[195] sequence for the synthesis of chaetomellic acid A and analogues. Unfortunately, despite many attempts, the addition-elimination reactions using a number of organocopper[196] reagents failed and only starting material was recovered. Reaction with dibromide **56** also failed to give any addition-elimination product. Nitrogen nucleophiles[197] also failed to add (Scheme 15). The failure of the addition-elimination reaction with organocopper species may be taken as evidence for the formation of the diene by-products *via* thermal and/or oxidative dimerization. In other words, the formation of dienes **54** and **55** can not be ascribed to coupling of the vinyl copper with **51** or **52**, but rather to thermal and/or oxidative dimerization (see Figure 24 above).


(CH3)2CuLi
CH3Cu(Me2S)•MgBr2
RCu (Me2S)•MgBrCl
R-NH2, base
CH3Cu(Me2S)•MgBr2
51
52
56
42 : R = n-C14H29
R = n-C14H29
OCH3
OBn
Br
H3C
CH3

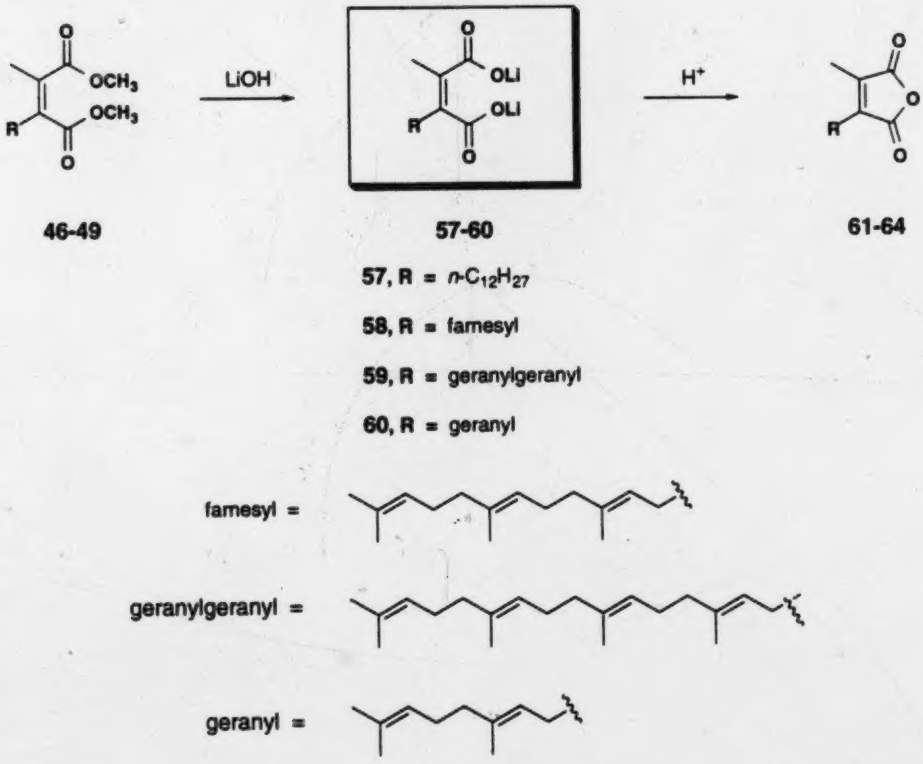
Scheme 15.

Vinyl stannane **53**, on the other hand, proved to be a useful synthon. Compound **53** was successfully coupled to farnesyl bromide under modified Stille conditions[198-200] using Pd/Cu to give the farnesyl derivative **47** in 85 % yield (Scheme 16). This established yet another route to **58** which turned out to be a potent inhibitor of PFTase (*vide infra*). Presumably this approach could also be extended to a variety of vinyl halides[201] to generate further analogues of chaetomellic acid A which may not be accessible through the direct conjugate addition/enolate trapping methodology.


farnesyl bromide
Pd(Ph3P)4 / CuI
DMF, 85%
OCH3
OCH3
(CH3)3Sn
53
47

Scheme 16.

Hydrolysis of **46-49** under conditions similar to those used for **42** generated the corresponding lithium salts **57-60**, which cyclized rapidly to the corresponding anhydrides **61-64** upon exposure to acid (Scheme 17).


LiOH
H+
OCH3
OCH3
OLi
OLi
R
46-49
57-60
61-64
57, R = n-C12H27
58, R = farnesyl
59, R = geranylgeranyl
60, R = geranyl
farnesyl =
geranylgeranyl =
geranyl =

Scheme 17.

2 Enzyme Inhibition.

Chaetomellic acid A di-lithium salt **44** and its analogues **57-59** were evaluated for inhibition of yeast protein farnesyltransferase (PFTase) and yeast protein geranylgeranyl-transferase-I (PGGTase-I) by professor Dale Poulter using the continuous fluorescence assay of Pompliano and co-workers.[202] The results are summarized in Table 5.

Table 5. Inhibition of protein prenyltransferases from yeast with chaetomellic acid **44** and analogues **57-59.**

	IC_{50} (µM)	
Inhibitor	PFTase	PGGTase-I
44	17 ± 3	>300
57	4 ± 0.1	112 ± 3
58	2.4 ± 0.08	277 ± 21
59	96 ± 16	11.5 ± 0.6

Chaetomellic acid A **44** inhibited yeast PFTase with an IC_{50} of 17 µM but did not inhibit PGGTase-I at all (IC_{50} >300 µM). Compound **57**, containing a side chain with two carbons less than the side chain of chaetomellic acid A, was a better inhibitor of yeast PFTase than chaetomellic acid A itself, with an IC_{50} of 4 µM, but it was less selective since it was also a weak inhibitor of PGGTase-I. Compound **58**, containing a farnesyl side chain, was the most potent inhibitor of PFTase and exhibited a good selectivity for PFTase over PGGTase-I (100:1). In contrast, analogue **59**, containing a geranylgeranyl side chain was a fairly good inhibitor of PGGTase-I (IC_{50} = 11.5 µM), although the level of selectivity for PGGTase-I over PFTase was lower (~10:1).

Compound **58** was shown to be a competitive inhibitor of PFTase (Figure 25) against FPP with a $K_I = 1.1 \pm 0.1$ μM. This pattern of inhibition is similar to that found for chaetomellic acid A when tested with PFTase from bovine brain.[119c]

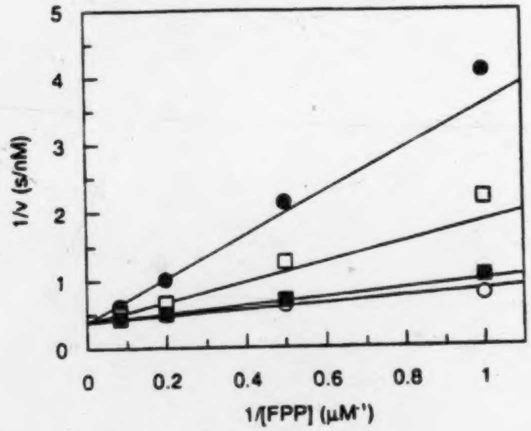
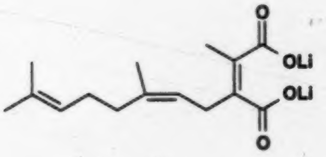


Figure 25. Inhibition of PFTase with analog **58**. Double-reciprocal plot with FPP as the varied substrate at fixed concentrations of **58**. Concentrations of analog **58** were 0.5 (○), 1 (■), 4 (☐), and 10 (●) μM. FPP was present at concentrations of 1-12 μM. Dansyl-Gly-Cys-Val-Ile-Ala was held constant at 2.4 μM. PFTase (1.1 nM) was used to initiate the reactions.

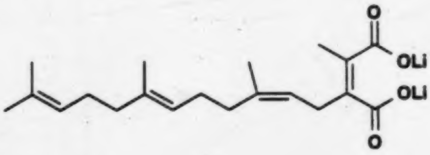
It is interesting to note the difference in potency of chaetomellic acid A as an inhibitor of PFTase from bovine brain and from yeast. Chaetomellic acid A has an IC_{50} of 55 nM with the enzyme from bovine brain and an IC_{50} of 17 μM with the yeast enzyme. This difference parellels the difference in the K_D values for FPP for the two enzymes. FPP binds much more tightly to PFTase from bovine brain (K_D = 12 nM)[203] than to the yeast enzyme (K_D = 75 nM).[204] An inhibitor which competes with FPP for binding might well be expected to reflect this difference. For example, α-(hydroxyfarnesyl)phosphonic acid, another competitive inhibitor with respect to FPP, has a reported IC_{50} of 30 nM with PFTase from bovine brain,[203] but a much higher IC_{50} (290 nM) with the yeast enzyme.

II Potential Inhibitors of *cis*-Prenyltransferases.

cis-Prenyltransferase mediates the sequential *cis*-addition of isopentenyl pyrophosphate (IPP) units, commencing with the addition of IPP to all-*trans*-FPP catalyzed by GGPP-synthetase to give *E, E, Z*-GGPP which is further transformed to polyprenyl-pp.[205] Recently, there has been interest in regulating the activity of GGPP-synthetase, and hence we designed compounds **67** and **76** to be tested against this enzyme.

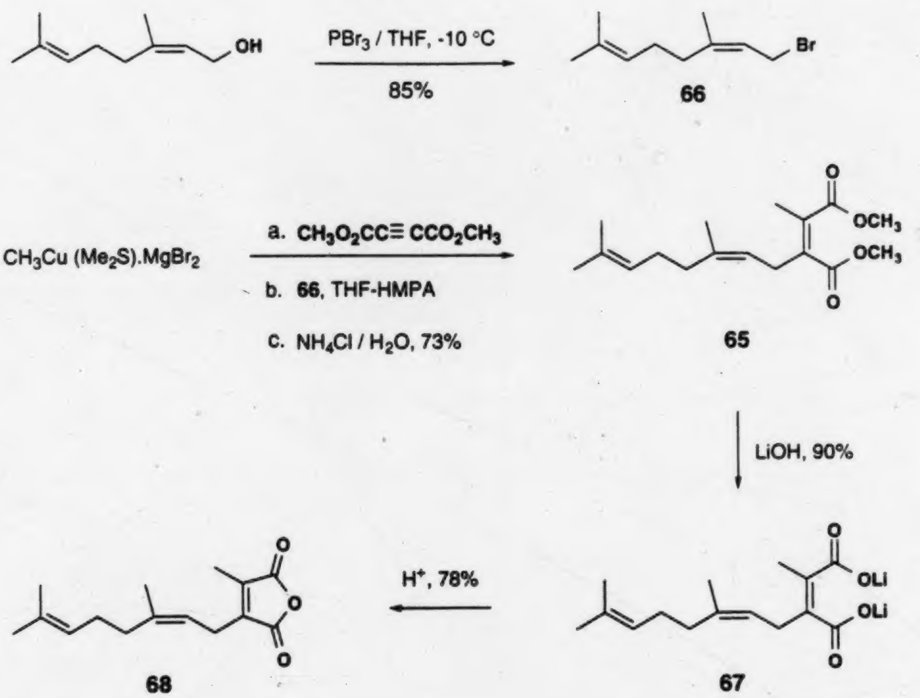


67



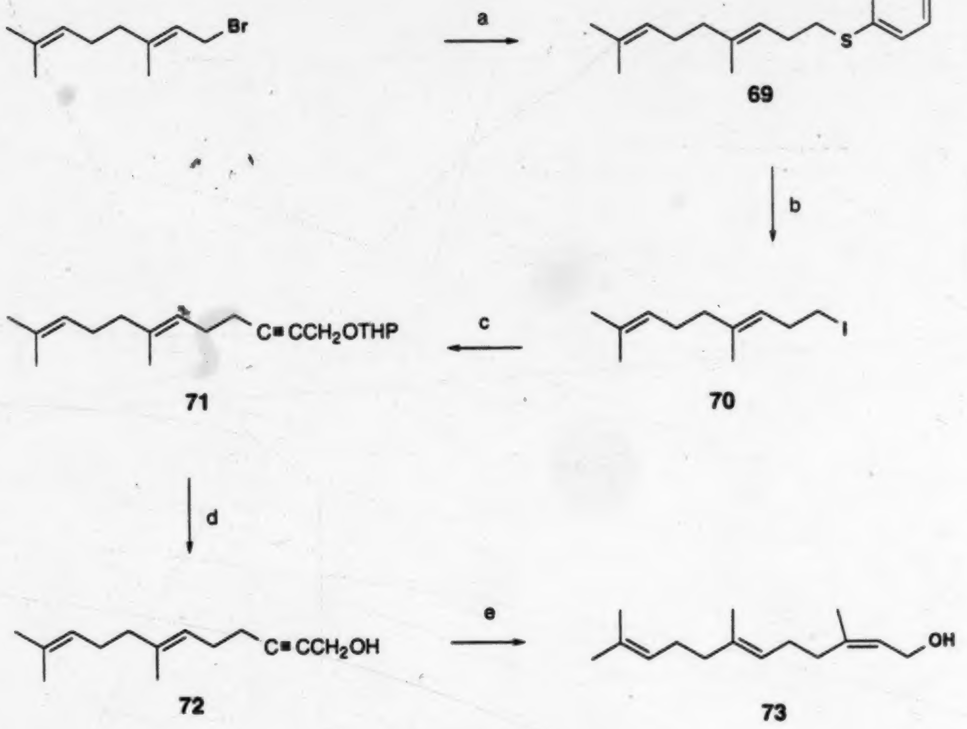
76

The synthesis of derivative **67** was straightforward and was based on the conjugate addition methodology developed above. Thus, alkylation of the conjugate addition adduct with neryl bromide **66** (freshly prepared from nerol) gave ester **65** in 73% yield. Base-catalyzed hydrolysis of **65** afforded the target compound **67** (90%), which could be cyclized to the corresponding anhydride **68** (78%), by exposure to acid (Scheme 18).


OH
PBr$_3$ / THF, -10 °C
85%
Br
66
CH$_3$Cu (Me$_2$S).MgBr$_2$
a. CH$_3$O$_2$CC≡CCO$_2$CH$_3$
b. 66, THF-HMPA
c. NH$_4$Cl / H$_2$O, 73%
O
OCH$_3$
OCH$_3$
O
65
LiOH, 90%
O
OLi
OLi
O
67
H$^+$, 78%
O
O
O
68

Scheme 18.

For the synthesis of the 2'-*Z*-farnesyl derivative **76**, 2-(*Z*)-6-(*E*)-farnesol had to be synthesized. The approach (Scheme 19) involved the transformation of the commercially avaliable geranyl bromide to homogeranyl iodide **70** over two steps and in 70% overall yield, using the method developed by Corey and co-workers.[206] Iodide **70** was then alkylated using the lithium derivative of tetrahydro-2-(2-propynyloxy)-2*H*-pyran in the presence of HMPA to give the acetylenic derivative **71** in 70% yield. The yield of this reaction was lower in the absence of HMPA. Removal of the THP protecting group gave propargyl alcohol **72** in quantitative yield. The critical step in the synthesis was the stereo- and regio-specific conversion of **72** to 2-(*Z*)-6-(*E*)-farnesol (**73**). The specific hydromagnesiation of alkynes developed by Sato and co-workers[207] seemed applicable here, and employing this methodology on **72** afforded the targeted farnesol **73** in 66% yield.


Br
a
S
69
b
I
70
c
C≡CCH$_2$OTHP
71
d
C≡CCH$_2$OH
72
e
OH
73

(a) PhSCH$_2$Li, CuI / THF, 90%;

(b) CH$_3$I, NaI / DMF, 77%;

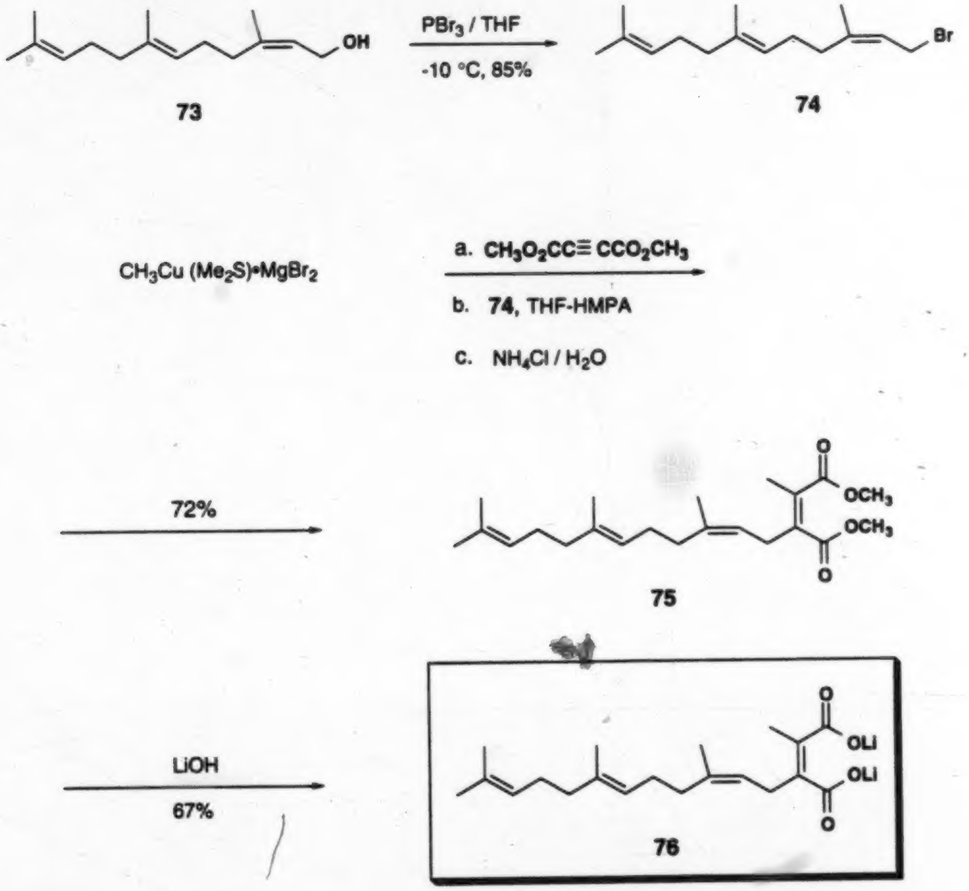
(c) LiC≡CCH$_2$OTHP/ HMPA, 70%;

(d) *p*-TsOH, CH$_3$OH, 99%;

(e) 2 ^{i}BuMgCl, then Cp$_2$TiCl$_2$, CH$_3$I, 66%.

Scheme 19

Having the required alcohol in hand, the synthesis of derivative **76** was completed by conversion of **73** to bromide **74**, which was then used to trap the conjugate addition adduct to give ester **75**. Careful hydrolysis of the ester afforded **76** in 19 % overall yield over 8 steps (Scheme 20). Compounds **67** and **76** are now being evaluated for biological activity.


OH
PBr3 / THF
-10 °C, 85%
Br
73
74
CH3Cu (Me2S)•MgBr2
a. CH3O2CC≡CCO2CH3
b. 74, THF-HMPA
c. NH4Cl / H2O
72%
O
OCH3
OCH3
O
75
LiOH
67%
O
OLi
OLi
O
76

Scheme 20.

Conclusions:

The present study[169] details methodology applicable for the rapid synthetic access to protein prenyltransferase inhibitors and demonstrates that modification of the side chain can greatly enhance both potency and selectivity for enzymes of this type. Furthermore, this study demonstrates that a dicarboxylic acid can act as a stable pyrophosphate mimic in biological systems.

CHAPTER 3 Inhibition of Peptidoglycan Biosynthesis

INTRODUCTION

1 Background.

Despite the great success of antibiotics over the last four decades, the worldwide emergence of bacterial strains resistant to current antibiotics has necessitated the development of new antimicrobial agents.[208,209] Due to the fact that the biosynthetic pathway which is used to produce the bacterial cell wall is non-existent in mammals, and to the vulnerability of bacteria to inhibition of this essential pathway, cell wall biosynthesis has been a focus for the development of antibiotics with high selectivity and low toxicity.[210,211] The bacterial cell wall is a complex structure composed of various macromolecules which provide much of the strength and rigidity, and function as an envelope to protect the delicate inner structure of the bacterial cell.[212] Among these macromolecules the major and most important constituent for the survival of the bacterial cell, is peptidoglycan. Defects or disruption of the peptidoglycan layer result in cell lysis and death of the bacteria.[213]

Peptidoglycan consists of a matrix of polysaccharide chains cross-linked through pentapeptide side chains. These peptide chains are attached to *N*-acetylmuramic acid (MurNAc) residues which alternate with *N*-acetylglucosamine (GlcNAc) to make up the polysaccharide backbone (Figure 26). The sequence of the pentapeptide is generally L-Ala-D-Glu-X-D-Ala-D-Ala, where X is usually *meso*-diaminopimelate (*m*-DAP) for Gram-negative bacteria, or L-lysine for Gram-positive bacteria.[214] Cross-linking of peptidoglycan chains occurs between the terminal amino group of residue X and the D-Ala residue of an adjacent peptide.[210]

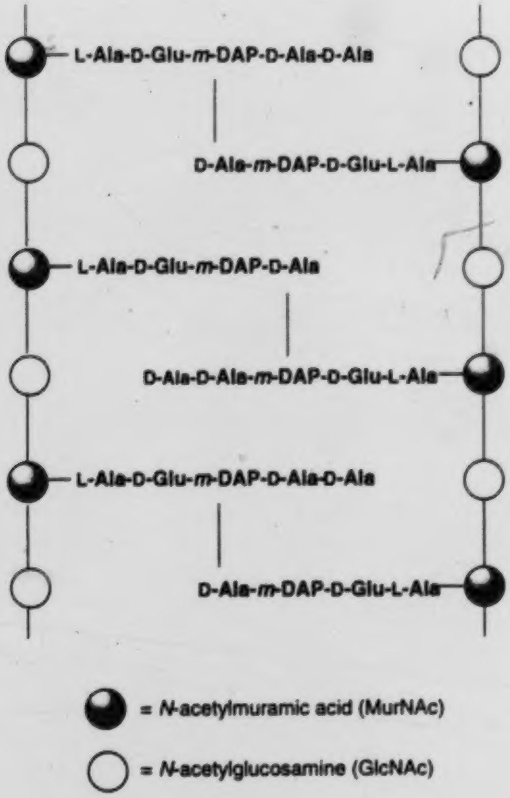

L-Ala-D-Glu-*m*-DAP-D-Ala-D-Ala
D-Ala-*m*-DAP-D-Glu-L-Ala
L-Ala-D-Glu-*m*-DAP-D-Ala
D-Ala-D-Ala-*m*-DAP-D-Glu-L-Ala
L-Ala-D-Glu-*m*-DAP-D-Ala-D-Ala
D-Ala-*m*-DAP-D-Glu-L-Ala
= *N*-acetylmuramic acid (MurNAc)
= *N*-acetylglucosamine (GlcNAc)

Figure 26. Schematic representation of the structure of the peptidoglycan layer in the cell wall of *E. coli.*

2 Biosynthesis of Peptidoglycan.

The biosynthesis of peptidoglycan can be divided into three stages. The synthesis of the cytoplasmic precursor UDPMurNac-L-Ala-D-Glu-*m*-DAP-D-Ala-D-Ala (UDPMurNAc-pentapeptide), the assembly of peptidoglycan monomer (PGM) units, and the polymerization of PGM.[210,212,213,215]

2.1 Synthesis of UDPMurNAc-Pentapeptide.

The pentapeptide precursor of peptidoglycan in *E. coli* is synthesized in the cytoplasm (Figure 27). *N*-Acetylglucosamine-1-phosphate (GlcNAc-1-P) is transformed to UDP-*N*-acetylmuramic acid (UDPMurNAc), a unique amino sugar found exclusively in the bacterial cell wall, over three steps. Firstly, UDP-*N*-acetylglucosamine (UDPGlcNAc) is formed *via* a reaction involving elimination of pyrophosphate (PPi) catalyzed by a transferase. Secondly, the nucleotide reacts with phosphoenolpyruvate, in a reaction catalyzed by a second transferase, to give the corresponding 3-enoylpyruvyl ether. Thirdly, reduction by a NADPH-dependent reductase produces UDPMurNAc. A stepwise addition of L-Ala, D-Glu and *meso*-DAP to UDPMurNAc, followed by addition of the D-Ala-D-Ala dipeptide, by a series of ligases, completes the synthesis of the precursor UDPMurNAc-pentapeptide.

2.2 Assembly of Peptidoglycan Monomer (PGM) Units.

The formation of PGM occurs on the cytoplasmic membrane. The UDPMurNAc-pentapeptide is transferred to the lipid carrier undecaprenyl phosphate by UDPMurNAc-pentapeptide phosphotransferase (translocase I).[216,217] A sugar transferase

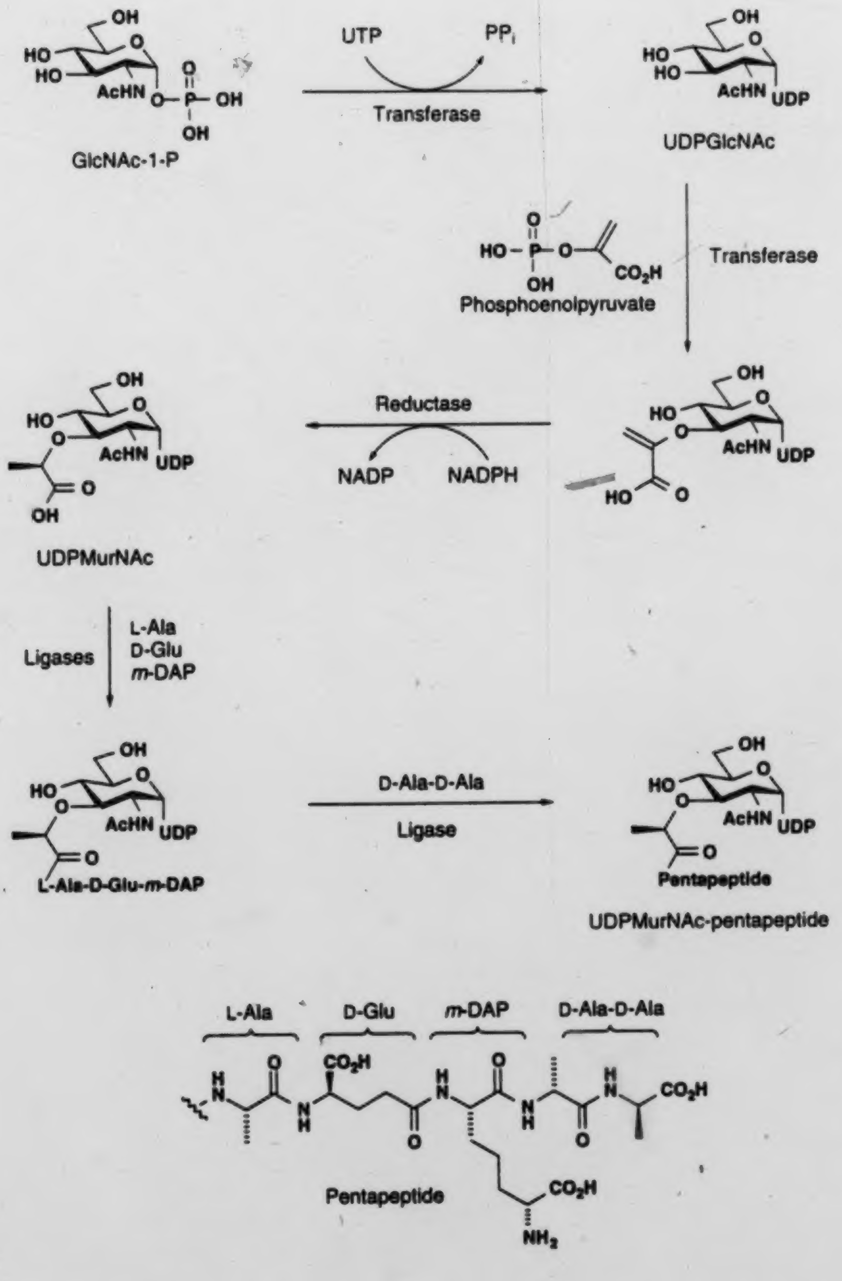

GlcNAc-1-P
UTP
PP$_i$
Transferase
UDPGlcNAc
Phosphoenolpyruvate
Transferase
Reductase
NADP
NADPH
UDPMurNAc
Ligases
L-Ala
D-Glu
m-DAP
L-Ala-D-Glu-m-DAP
D-Ala-D-Ala
Ligase
Pentapeptide
UDPMurNAc-pentapeptide
L-Ala
D-Glu
m-DAP
D-Ala-D-Ala
Pentapeptide

Figure 27. The synthesis of the precursor UDPMurNAc-pentapeptide.

(translocase II) then catalyzes a glycosidation reaction in which an *N*-acetylglucosamine residue is added to form the complete PGM unit (Figure 28).[218]

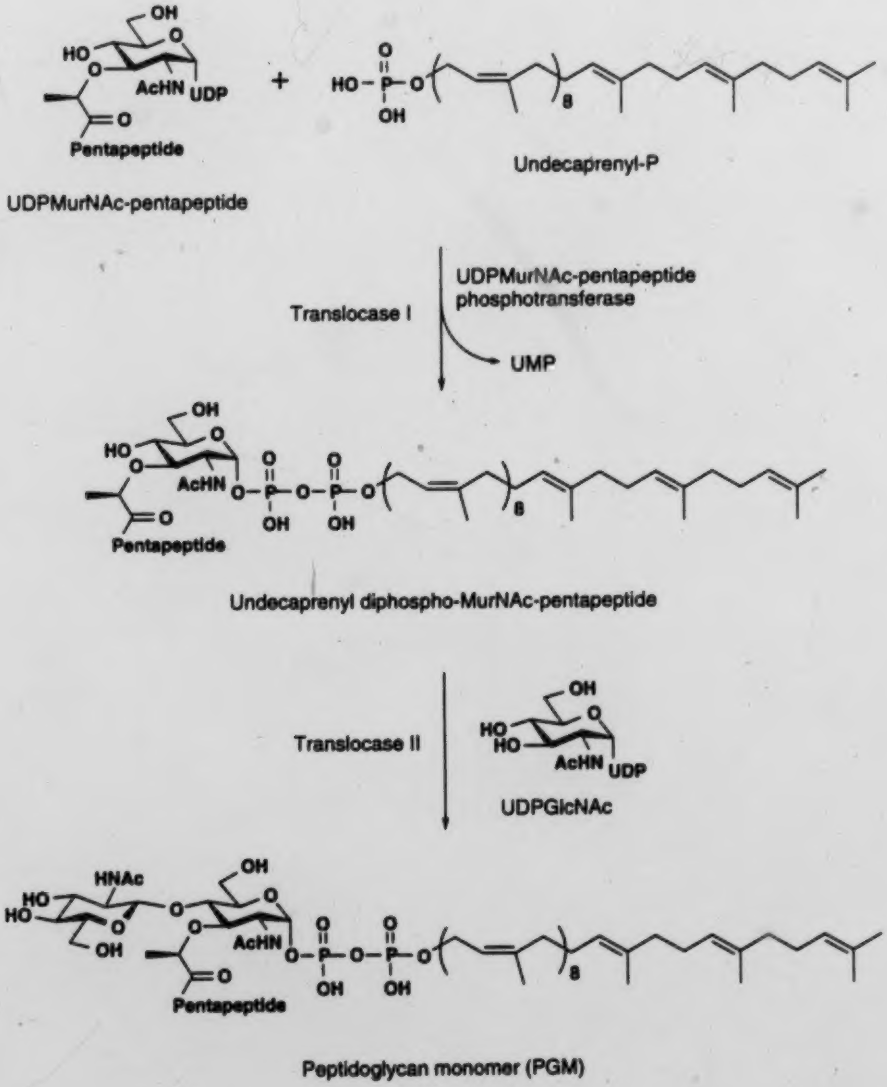

UDPMurNAc-pentapeptide
Pentapeptide
Undecaprenyl-P
Translocase I
UDPMurNAc-pentapeptide phosphotransferase
UMP
Undecaprenyl diphospho-MurNAc-pentapeptide
Translocase II
UDPGlcNAc
Peptidoglycan monomer (PGM)

Figure 28. The assembly of the peptidoglycan monomer (PGM).

2.3 Polymerization of PGM.

In this stage, the PGM is transferred to the outer face of the cytoplasmic membrane where polymerization occurs through transglycosylation, then cross-linking is acheived by transpeptidation processes.

The transglycosylation process involves the formation of a β-1,4-glycosidic linkage between an *N*-acetylmuramic acid residue of the PGM and the terminal *N*-acetylglucosamine residue of the growing polysaccharide chain (Figure 29). Thus, the growth of the peptidoglycan chain takes place by successive addition of disaccharide units.

The transglycosylation reaction releases undecaprenyl pyrophosphate, which is recycled *via* dephosphorylation to undecaprenyl phosphate to be used in another cycle (see Figure 28). The transpeptidation reaction cross-links the glycan units through two peptide units.

3 Natural Inhibitors of the Transglycosylase Reaction.

A number of bifunctional enzymes, known as penicillin binding proteins (PBPs), have been found to catalyze both transglycosylation and transpeptidation reactions.[219,220] Penicillin and other β-lactam antibiotics were found to covalently bind to the active sites of these enzymes, thereby inhibiting their function, which eventually leads to cell lysis. A large number of naturally occurring antibiotics are known to function by inhibition of various stages of peptidoglycan biosynthesis and many of them, such as β-lactams (e.g penicillin) and glycopeptides (e.g vancomycin), are used widely in clinical situations.[210]

A unique class of antibiotics, the phosphoglycolipids,[221] is believed to have a mechanism based on the selective inhibition of the transglycosylation step.[222]

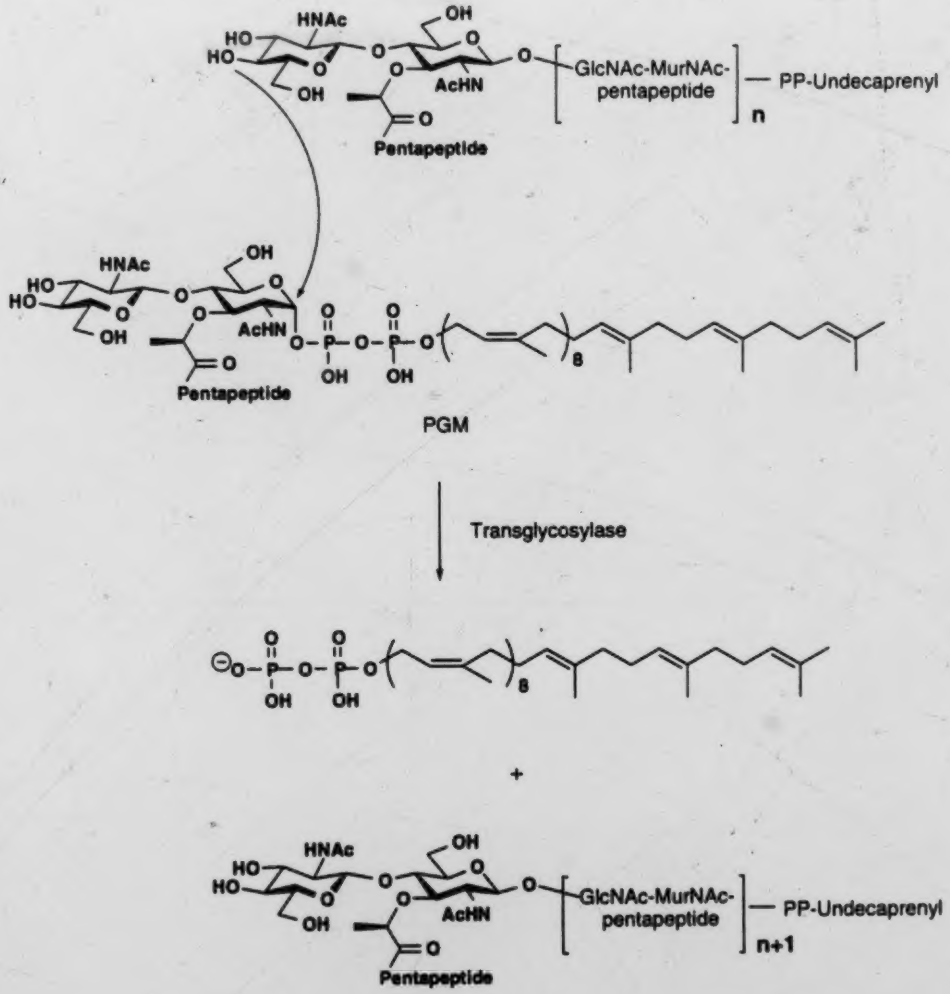

HNAc
OH
HO
HO
O
AcHN
Pentapeptide
GlcNAc-MurNAc-pentapeptide
PP-Undecaprenyl
n
8
PGM
Transglycosylase
+
n+1

Figure 29. The transglycosylation reaction.

Phosphoglycolipid antibiotics include moenomycin, ensachomycin, prasinomycins, marcarbomycins, teichomycin, quebemecin, prenomycin, and pholipomycin which are all produced by various species of *Streptomyces.*[221] Of the moenomycin-type compounds, moenomycin A (Figure 30), first reported in 1965,[223] is the main component of the trade product Flavomycin®.

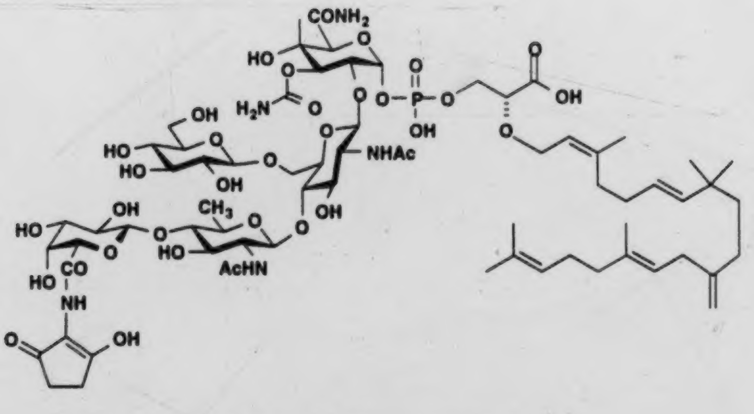

CONH₂
O
HO
O
O
O-P-O
OH
OH
H₂N
O
OH
O
NHAc
HO
HO
OH
CH₃
OH
HO
O
HO
AcHN
HO
CO
NH
O
OH

Figure 30. Moenomycin A.

Studies with cell-free systems from *E. coli* have demonstrated that moenomycin A selectively inhibits the transglycosylation step by its inhibitory effect on PBP 1b at concentrations between 10^{-8} and 10^{-7} M.[224] It has been speculated that moenomycin A interacts with the enzyme (PBP 1b) because of its structural similarity with the membrane bound GlcNAc-MurNAc-(pentapeptide)-PP-undecaprenol (PGM) mentioned above.[225] Structure-activity relationship studies have given support for this view, and systematic degradations have shown that only a portion of the moenomycin A structure is essential for antibiotic activity.[224a] In fact, the disaccharide derivative shown (Figure 31) retains the full antibiotic activity of the parent compound.[226]

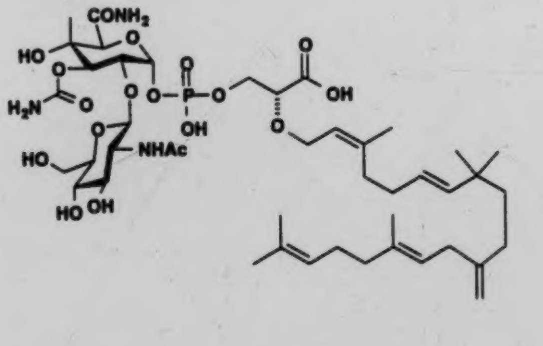

CONH₂
O
HO
O
O
O-P-O
OH
OH
H₂N
O
O
NHAc
HO
HO
OH

Figure 31. Active portion of Moenomycin A.

Recently, the antibiotic moenomycin C_1 was reported to be an inhibitor of transglycosylase.[227] In this series, the minimum structure required for the full antibiotic activity is the trisaccharide derivative shown (R = H, Figure 32).

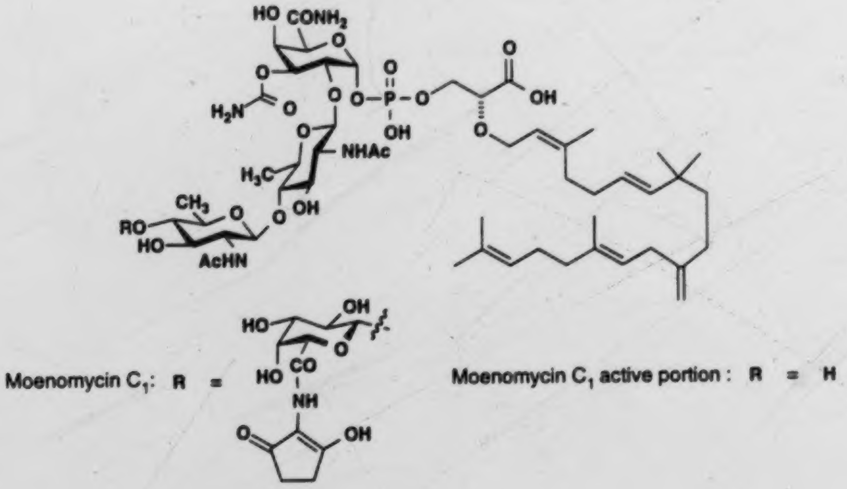

HO CONH₂
O
O
H₂N
O
O
O–P–O
OH
O
OH
NHAc
H₃C
OH
CH₃
RO
HO
AcHN
O
OH
HO
HO
CO
NH
O
OH
Moenomycin C₁: R =
Moenomycin C₁ active portion : R = H

Figure 32. Moenomycin C_1 and the active portion.

4 The Design of Transglycosylase Inhibitors.

Degradative studies have suggested that other small, synthetically accessible molecules could be inhibitors of transglycosylase. Several small, synthetic analogues of moenomycins A and C_1, which are biologically active, have appeared in the literature.[228] It has been reported that the monosaccharide degradation product shown in Figure 33 retains some of the biological activity of moenomycin A in both an enzyme assay and in antibacterial testing.[229]

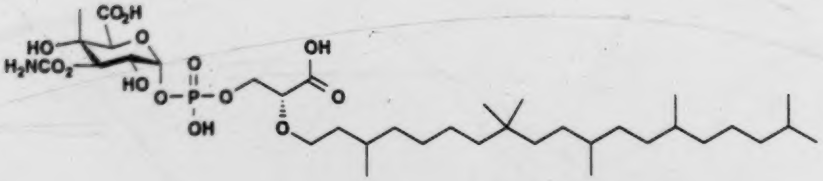


Figure 33. Biologically active monosaccharide degradation product.

This encouraged investigation of the hypothesis that the enzyme may be inhibited by monosaccharide derivatives such as glucose or *N*-acetylglucosamine, linked by a pyrophosphate surrogate to a lipid-like component. Based on the structure of peptidoglycan monomer (PGM), a chitobiose derivative could also retain recognition elements required by the enzyme. Since the maleic acid moiety proved a good pyrophosphate mimic in the design of PFTase inhibitors, it was decided to use it to link the above mentioned carbohydrate derivatives to the lipid.

Three types of targets were designed, as shown in Figure 34. Type A was designed to explore the possibility of using the non-cleavable *C*-glycoside as a stable surrogate for the *O*-glycoside, and to probe the distance requirements between the sugar and the diacid moieties. Type B is similar to the degradation product shown in Figure 33, having the diacid moiety mimicking the phosphoglycerate anionic group. Type C targets were designed based on the structure of the natural substrate, peptidoglycan monomer (PGM), of the transglycosylase. The GlcNAc derivative was targeted to examine the importance of the second sugar of PGM. The chitobiose derivative closely resembles PGM.

In addition to potentially inhibiting the transglycosylase, these compounds could also "end-cap" the growing polysaccharide chain if they are incorporated into peptidoglycan. Furthermore, analogues which are structurally similar to peptidoglycan monomer may be useful as probes with respect to the transglycosylase active site and mechanism of reaction, and they are likely to have better physical properties, such as water solubility, than moenomycin-based analogues.

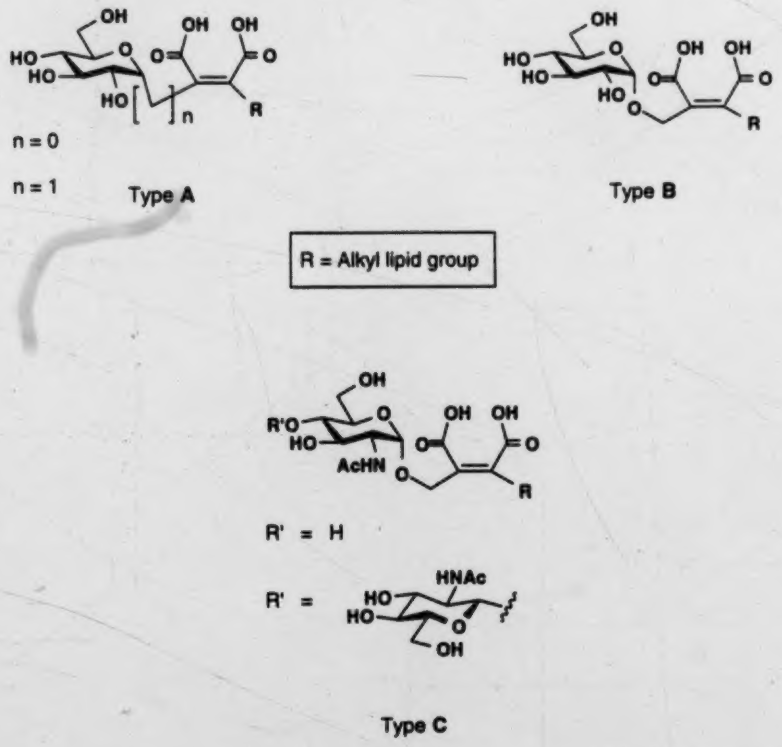

n = 0
n = 1
Type A
Type B
R = Alkyl lipid group
R' = H
R' =
Type C

Figure 34. Synthetic target molecules.

RESULTS AND DISCUSSION

1 Synthetic Studies on the Type A Targets: *C*-glycosides.

The synthetic strategy for the construction of type **A** (n = 0) inhibitors was based on the retrosynthetic analysis outlined in Figure 35. The target molecule seemed accessible from the maleate derivative **40a**, already carrying the lipid chain, and acetobromo glucose *via* a radical coupling approach. Further elaboration of the coupling adduct to generate the double bond, followed by deprotection, should furnish the target compound.

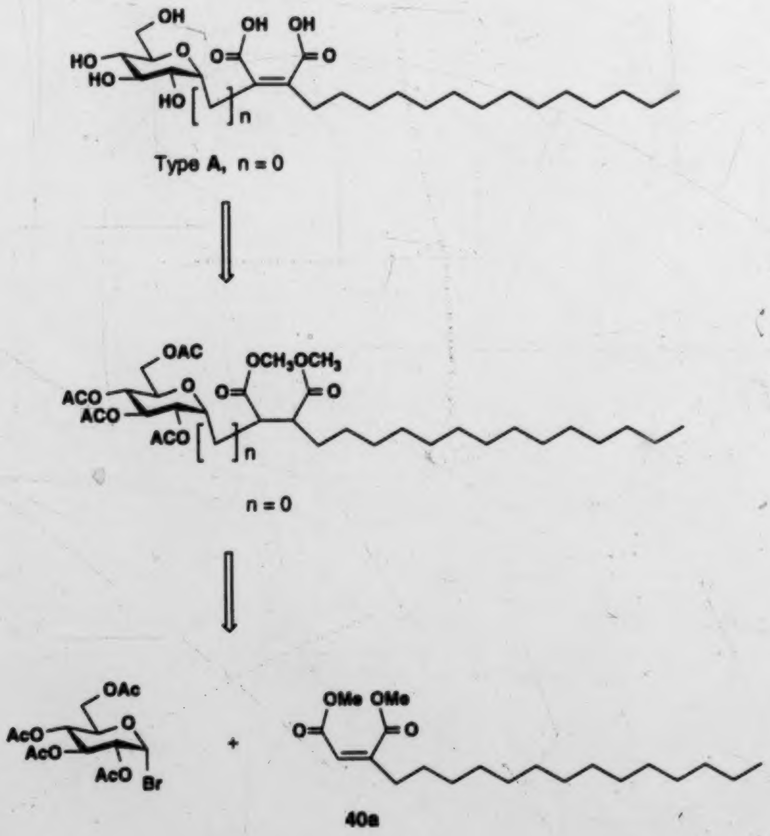
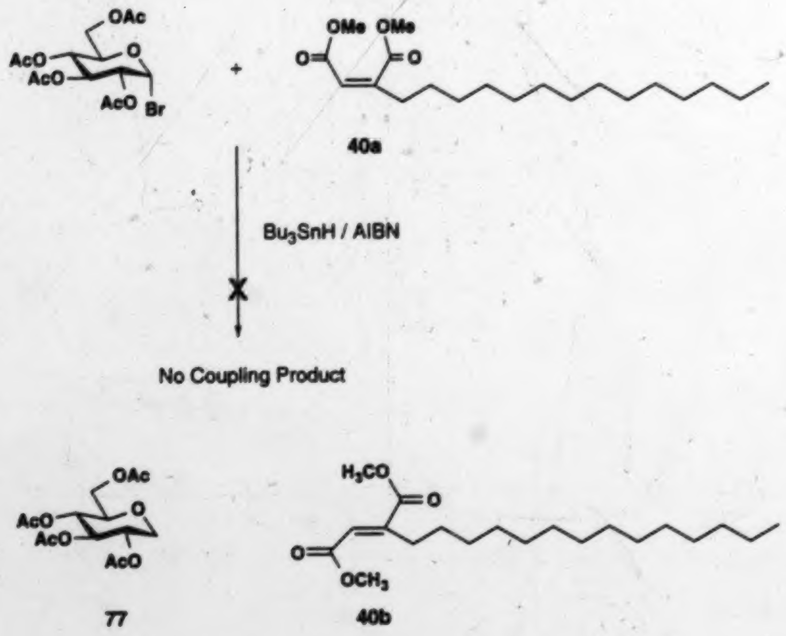

OH
HO
HO
O
HO
OH OH
O
O
n
Type A, n = 0
OAC
ACO
ACO
O
ACO
OCH3OCH3
O
O
n
n = 0
OAc
AcO
AcO
O
AcO
Br
+
OMe OMe
O
O
40a

Figure 35.

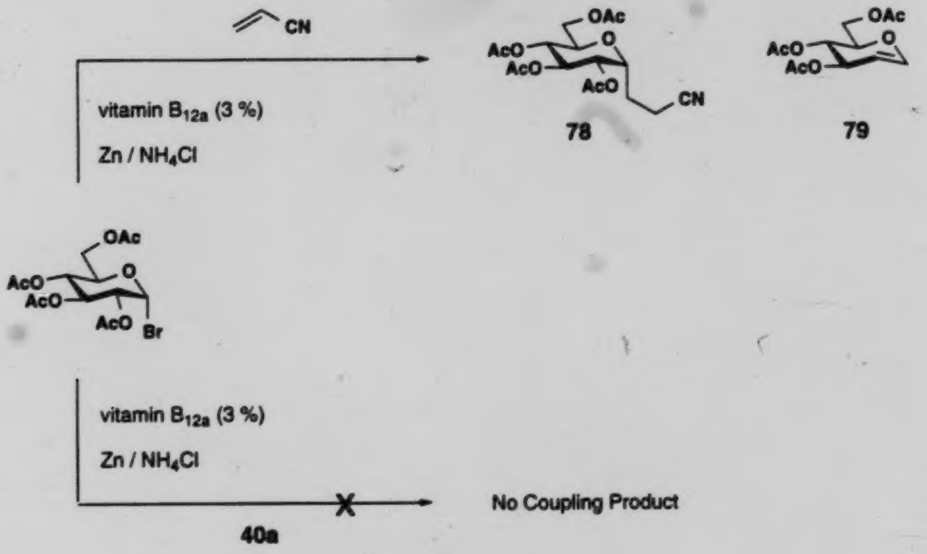
The idea was not without merit since we already had access to compound **40a** and acetobromo glucose is commercially available. The approach also seemed attractive since glycosyl radicals are known to give predominantly α-*C*-glycosides.[230,231]

However, when we tried the reaction between acetobromo glucose and maleate **40a** in the presence of *n*-Bu_3SnH and AIBN, no coupled product was formed despite the use of different reaction conditions (varying reaction time, temperature, solvent and concentration of *n*-Bu_3SnH). Careful analysis of the reaction mixtures indicated that they contained starting materials along with the isomerized maleate **40b** and what seemed to be the reduced product 1,5-anhydro D-glucitol **77** as indicated by MS and NMR spectroscopy (Scheme 21). It appears that abstraction of the bromine atom by *n*-$Bu_3Sn^•$ is slower than the addition of *n*-$Bu_3Sn^•$ to maleate **40a**, which presumably caused the isomerization to **40b**.



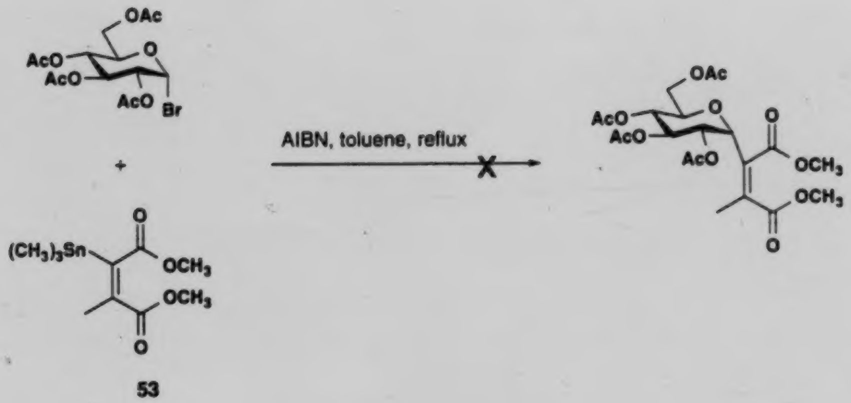
Scheme 21.

An alternative approach for the generation of glycosyl radicals is to use vitamin B_{12}. Vitamin B_{12}, a coenzyme known to promote a series of biological transformations *via* radical intermediates[232], has been used as a catalyst for carbon-carbon bond formation *via* radicals.[233] Thus, when acetobromo glucose was reacted with acrylonitrile in the presence of a catalytic amount of vitamin B_{12a}, the desired *C*-glycoside **78** was formed in 43% yield along with the glucal **79**, which was produced in 40% yield[230a] (Scheme 22). The same reaction with **40a** failed to give any coupled product.


CN
OAc
AcO
AcO
AcO
O
CN
78
OAc
AcO
AcO
O
79
vitamin B12a (3 %)
Zn / NH4Cl
OAc
AcO
AcO
O
AcO
Br
vitamin B12a (3 %)
Zn / NH4Cl
No Coupling Product
40a

Scheme 22.

Baldwin[234] and Russell[235] have shown that a variety of radicals react with vinyl stannane to give the product of an addition-elimination reaction. Based on these results, we tried the radical addition-elimination reaction on vinyl stannane **53** (Scheme 23). Unfortunately, in our case no coupled product was formed.


OAc
AcO
AcO
O
AcO
Br
+
(CH3)3Sn
OCH3
OCH3
53
AIBN, toluene, reflux
OAc
AcO
AcO
O
AcO
OCH3
OCH3

Scheme 23.

We next turned to the other compound of the *C*-glycoside targets (Type **A**, n = 1). A retrosynthetic analysis revealed that the target molecule may be accessible *via* the conjugate addition-enolate trapping methodology developed above. Thus, conjugate addition of an organo copper reagent carrying the lipid chain to DMAD, followed by capture of the vinyl copper adduct with the appropriate sugar electrophile should build up the complete skeleton in a single step (Figure 36).

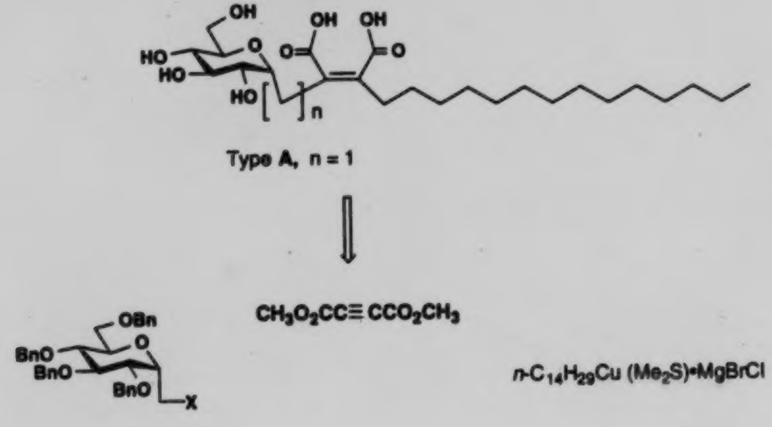
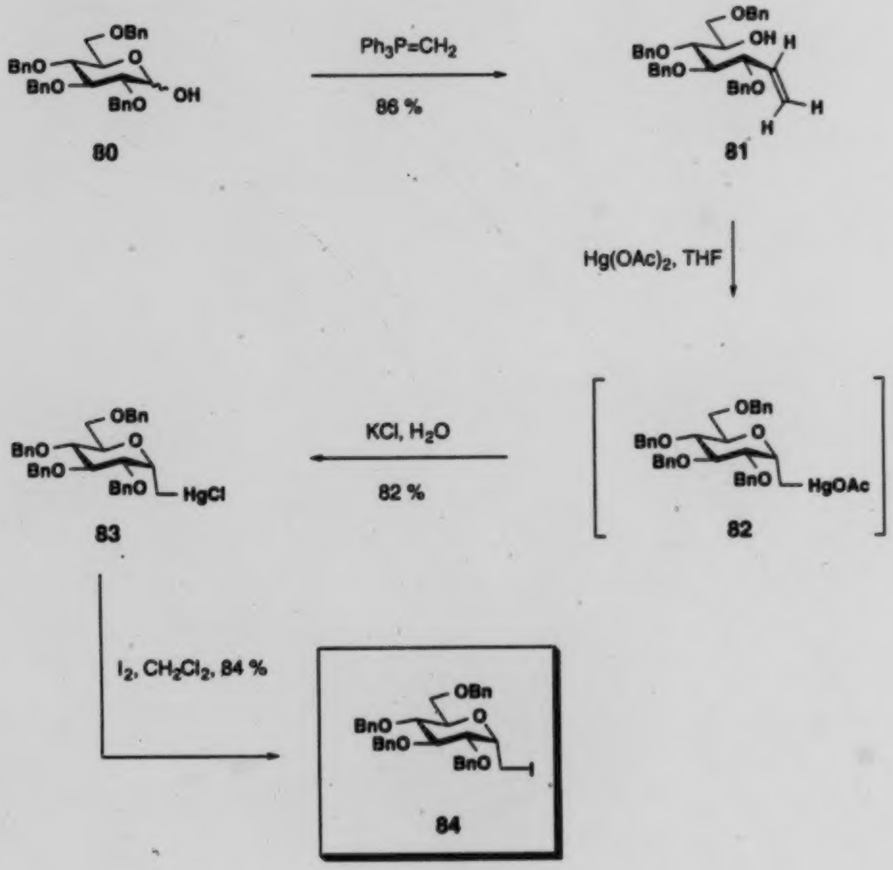

OH
OH OH
HO
HO
HO
O
n
Type A, n = 1
OBn
BnO
BnO
BnO
X
CH3O2CC≡CCO2CH3
n-C14H29Cu (Me2S)•MgBrCl

Figure 36.

The required methylene iodide *C*-glycoside **84** was prepared as shown in Scheme 24. Wittig olefination[236] on the commercially available 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose **80** using methylene triphenylphosphorane afforded olefin **81**. Mercury-assisted cyclization[237] of **81** selectively provided the α-*C*-glycoside as an unstable mercury acetate **82**, which was stabilized by conversion to the mercury chloride **80** using aqueous potassium chloride. Iodomercuration of **83** provided the target methylene iodide *C*-glycoside **84** in 59 % overall yield.


OBn
O
BnO
BnO
BnO
OH
80
Ph₃P=CH₂
86 %
OBn
OH
H
BnO
BnO
BnO
H
H
81
Hg(OAc)₂, THF
OBn
O
BnO
BnO
BnO
HgOAc
82
KCl, H₂O
82 %
OBn
O
BnO
BnO
BnO
HgCl
83
I₂, CH₂Cl₂, 84 %
OBn
O
BnO
BnO
BnO
I
84

Scheme 24.

The observed facial diastereoselectivity of the cyclization reaction is believed to be caused by the suitable orientation of the 2-*O*-benzyl group which, exerts a strong directing effect by co-ordinating with the incoming mercury species (Figure 37).

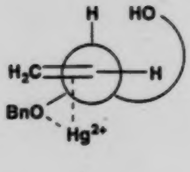
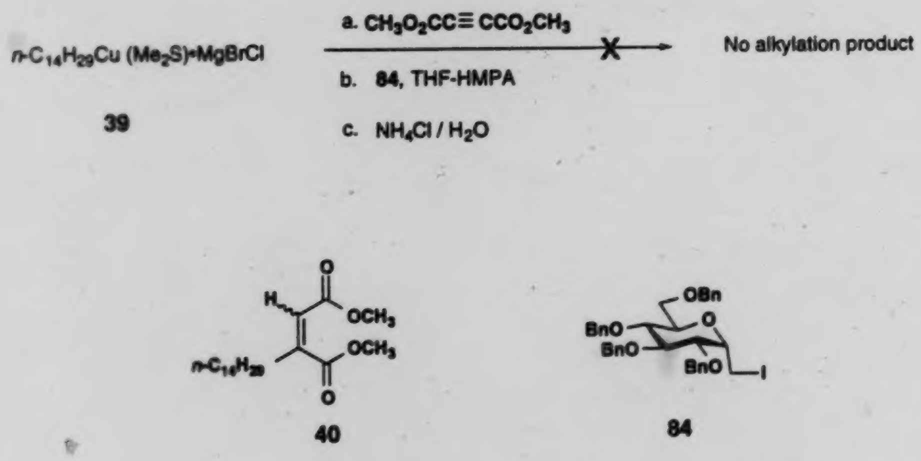

H
HO
H_2C
H
BnO
Hg^{2+}

Figure 37.

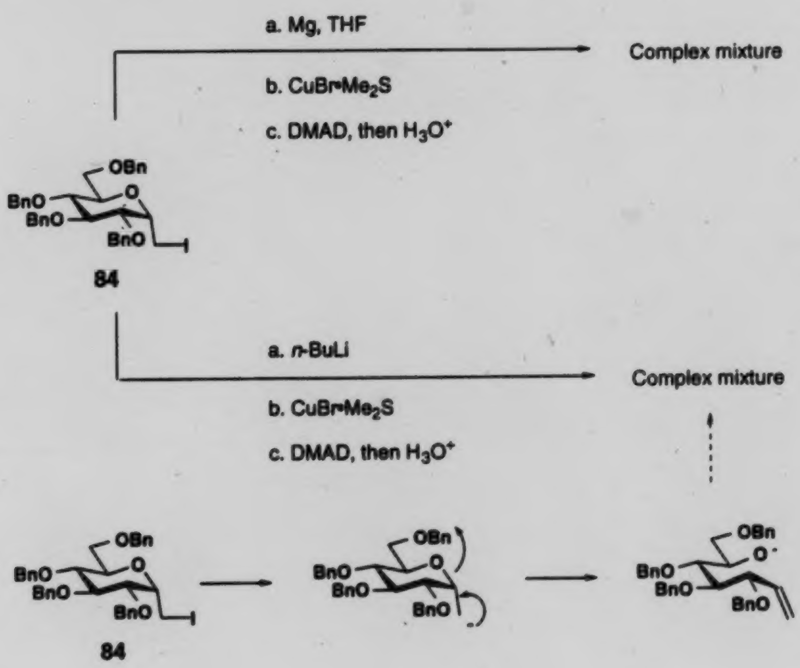
Having made the required precursor **84**, we then proceeded to examine the conjugate addition/enolate trapping reaction (Scheme 25). Thus, conjugate addition to DMAD as before, followed by addition of *C*-glycoside **84**, failed to give any of the alkylation product and instead **40** , resulting from protonation of the vinyl copper intermediate, was obtained. Most of the starting material **84** was also recovered.


$n\text{-}C_{14}H_{29}Cu\ (Me_2S)\bullet MgBrCl$
39
a. $CH_3O_2CC\equiv CCO_2CH_3$
b. 84, THF-HMPA
c. NH_4Cl / H_2O
No alkylation product
H
O
OCH_3
OCH_3
$n\text{-}C_{14}H_{29}$
O
40
OBn
O
BnO
BnO
BnO
I
84

Scheme 25.

Apparently, **84** is too sterically hindered to alkylate the vinyl copper adduct. Therefore, we planned to reverse the order of addition, i.e., first conjugate addition of an organo copper reagent derived from **84**, followed by enolate capturing with tetradecyl bromide. Before doing so however, the conjugate addition using **84** was first examined. Unfortunately, attempted conjugate addition of organo copper reagents derived from **84** followed by protonation resulted in only complicated mixtures (Scheme 26).

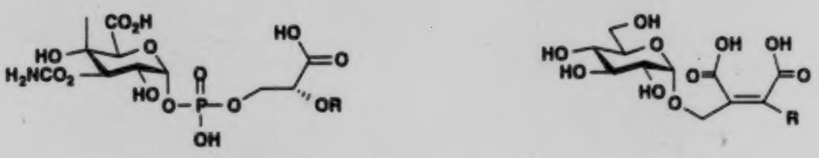
Although the above approaches were unsuccessful, they provided insight into the behavior of some of the precursors involved. The lack of success of these approaches also demonstrated the relative level of difficulty that could be encountered when synthesizing *C*-glycosides compared to *O*-glycosides. Furthermore, it became apparent that the *C*-glycoside targets mentioned above may be better synthesized by step-wise approaches rather than convergent ones.


a. Mg, THF
b. CuBr•Me$_2$S
c. DMAD, then H$_3$O$^+$
Complex mixture
OBn
BnO
BnO
BnO
O
I
84
a. n-BuLi
b. CuBr•Me$_2$S
c. DMAD, then H$_3$O$^+$
Complex mixture
84
O$^-$

Scheme 26.

2 Synthetic Studies on the Type B Targets.

The type **B** target molecules are structurally similar to the monosaccharide degradation product of moenomycin A which retains some biological activity (Figure 38). Structural features in the type **B** target include a diacid moiety, mimicking the phophoglycerate anionic group, a lipid chain and the sugar moiety for recognition.



Moenomycin A degradation product

Type **B** target

R = Lipid alkyl group

Figure 38.

A retrosynthetic analysis (Figure 39) of the type **B** target compound indicated that the target molecule should be accessible *via* an *O*-glycosylation reaction[238] between the glycosyl acceptor, hydroxymethyl maleate derivative **89**, and a suitably protected glycosyl donor. The required hydroxymethyl derivative **89** seemed attainable using the conjugate addition/enolate trapping methodology developed previously.

Preliminary computer modeling showed that both the α- and β-anomers of the type **B** target compound occupy approximately the same space, presumably due to the flexibility of the linkage between the two rigid moieties present in the molecule, namely the maleic acid and the glucose ring. However, to keep close resemblance to the natural inhibitors, the α-anomer was initially sought. This makes the choice of the protecting groups on the glycosyl donor important (*vide infra*).

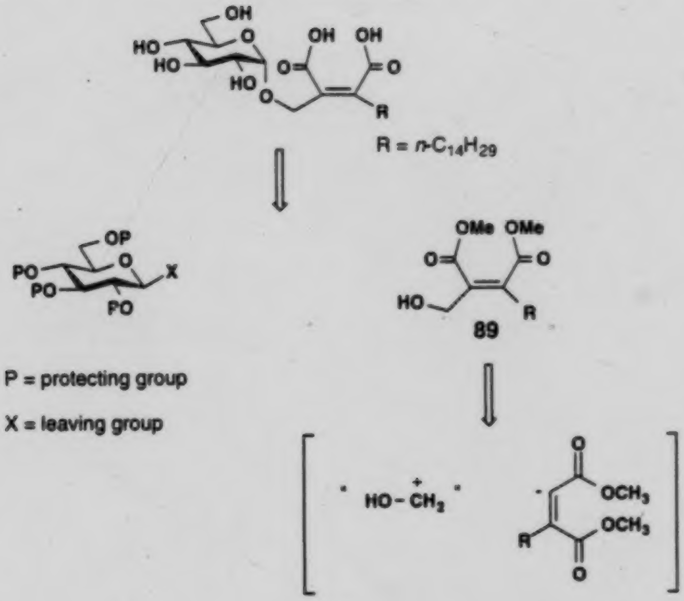
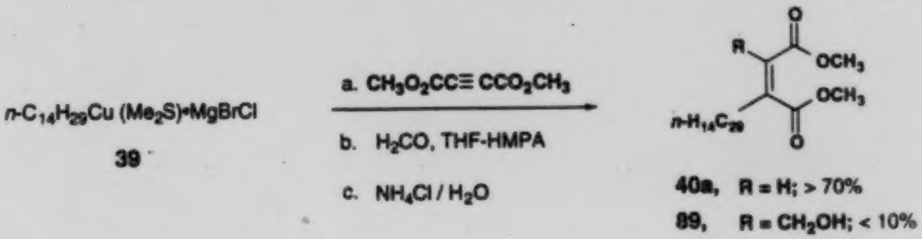

R = n-C14H29
P = protecting group
X = leaving group
89

Figure 39.

2.1 Synthesis of the glycosyl acceptor 89.

Using the conjugate addition-enolate trapping methodology developed above, condensation of the conjugate addition adduct with formaldehyde (Scheme 27) seemed to be the most attractive route to **89**. Unfortunately, despite several attempts, the condensation product **89** was formed only in low yields (< 10%), and the major product

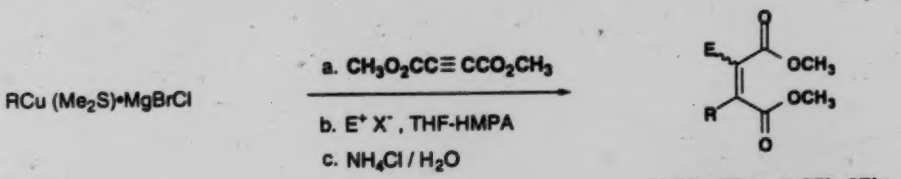

n-C14H29Cu (Me2S)•MgBrCl
39
a. CH3O2CC≡CCO2CH3
b. H2CO, THF-HMPA
c. NH4Cl / H2O
40a, R = H; > 70%
89, R = CH2OH; < 10%

Scheme 27.

was the conjugate addition adduct **40a**. The inefficiency of this reaction, which was expected to proceed in a very straightforward manner, was probably due to practical limitations since it was very difficult to pass the formaldehyde gas into the thick, heterogeneous reaction mixture.

The second, less direct route to **89** was based on alkylation of the conjugate addition adduct by α-haloethers ($ROCH_2X$).[239] Three types of these protected hydroxymethylating agents were examined, namely methoxymethyl chloride (MOMCl), benzyloxymethyl chloride (BOMCl) and trimethylsilylethoxymethyl chloride (SEMCl). Table 6 presents unoptimized yields and stereoselectivity ratios from preliminary investigations of the alkylating ability of these electrophiles. These reactions could potentially be improved by conducting the alkylation of the conjugate addition adduct at lower temperatures.

Table 6. Conjugate addition to DMAD and capture with α-haloethers.

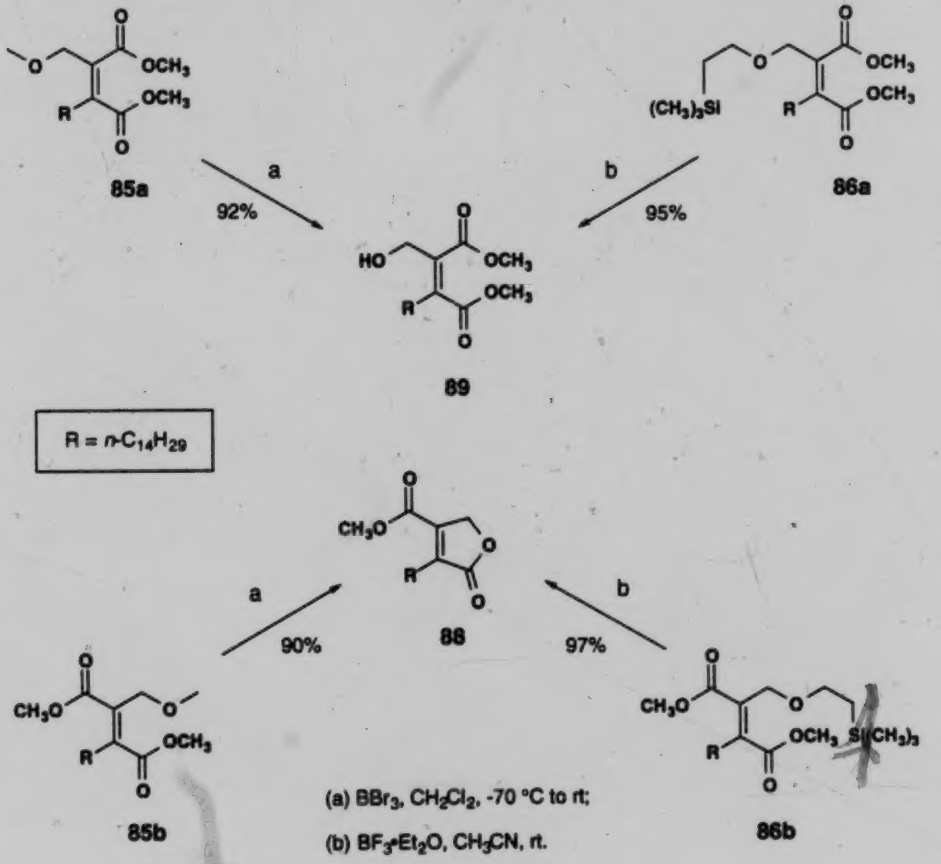


Z (**85a-87a**), *E* (**85b-87b**)

Entry	R	$E^+ X^-$	E	Product (ratio)[a]	Yield (%)[b]
1	n-$C_{14}H_{29}$	MOMCl	CH_3OCH_2-	**85a:85b** (1 : 2.8)	73
2	n-$C_{14}H_{29}$	SEMCl	$(CH_3)_3SiCH_2OCH_2$-	**86a:86b** (1 : 1.9)	82
3	CH_3	BOMCl	$BnOCH_2$-	**87a:87b** (0 : 1.0)	32

[a] Ratio of isolated materials. [b] Isolated yields

The stereochemical assignment for these tetrasubstituted alkenes was based on ^{1}H NMR chemical shift arguments and chemical derivatization. In all three cases the ^{1}H NMR chemical shift of the allylic substituent *cis* to the ester group is more downfield than when it is *cis* to the hydroxymethyl group. This is in agreement with what was found for those similar compounds reported in the previous chapter. Regarding chemical derivatization as evidence for the stereochemical assignment, lactone **88** was obtained from both **85b** and **86b** after deprotection, while the hydroxymethyl maleate **89**, derived from **85a** and **86a**, remained unchanged for obvious geometrical reasons (Scheme 28).

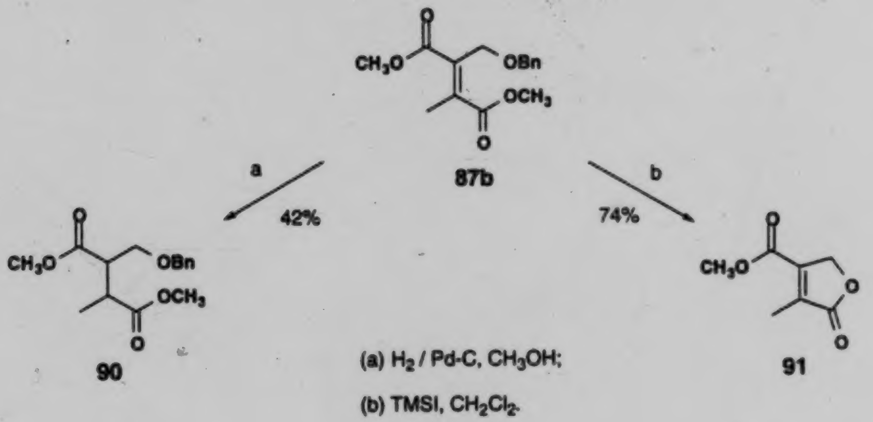


(a) BBr$_3$, CH$_2$Cl$_2$, -70 °C to rt;

(b) BF$_3$•Et$_2$O, CH$_3$CN, rt.

Scheme 28.

Derivative **87b** was obtained in low yield partly because a low quality sample of BOMCl was used. Despite the manufacturer's claim that their BOMCl sample was at least 95 % pure, it was found later that this sample actually contained major contaminants such as benzyl alcohol, which is used as the starting material for preparing BOMCl,[240] as well as benzyl chloride and formaldehyde dibenzylacetal, which are likely by-products in its preparation. Even though it was the *E*-isomer, compound **87b** was used as a model to investigate if the benzyl protecting group could be removed selectively in the presence of the double bond. Attempted removal of the benzyl group by hydrogenation resulted in the reduction of the double bond, and after 15 min **90** was produced in 42 % yield along with the remaining unreacted starting material. Specific removal of the benzyl group in **87b** using trimethylsilyl iodide[241] afforded the expected lactone **91** (Scheme 29).



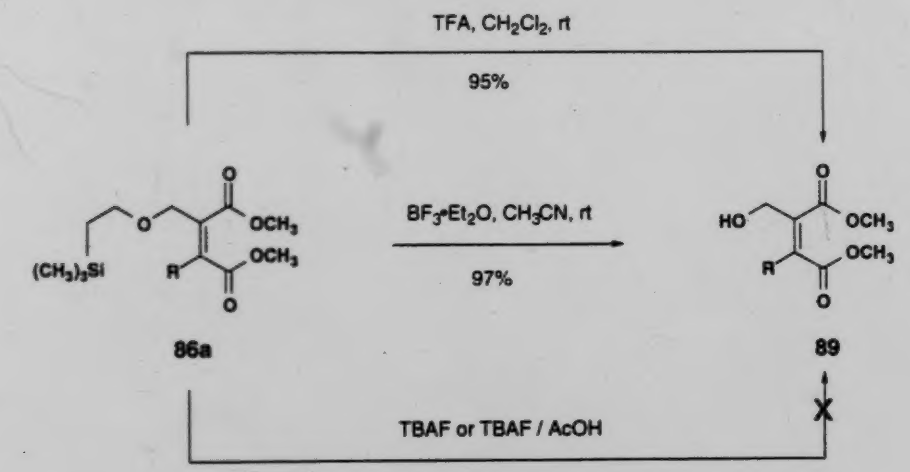
(a) H_2 / Pd-C, CH_3OH;

(b) TMSI, CH_2Cl_2.

Scheme 29.

For the synthesis of the required hydroxymethyl maleate derivative **89**, we focused on the use of the SEMCl reagent since it gave the best chemical yield and stereochemical ratio. Three methods were examined for the deprotection of **86a**

(Scheme 30) in order to find optimal conditions for recovery of the alcohol. The best conditions were found to be $BF_3{\cdot}Et_2O$ in CH_3CN for 30 min.



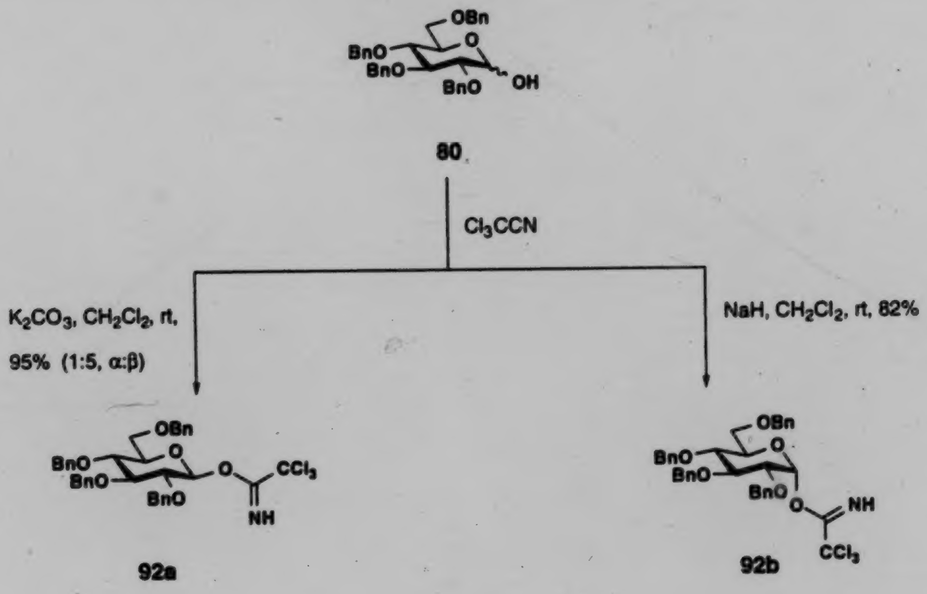
Scheme 30.

2.2 Synthesis of the glycosyl donor.

Having secured a large amount of the required glycosyl acceptor **89**, we turned to the synthesis of the glycosyl donor (see Figure 39). In our synthetic strategy we sought to utilize the imidate methodology developed by Schmidt.[242] In order to achieve the glycosylation coupling with the desired α-stereochemistry, two requirements had to be met. Firstly, the glycosyl donor must have a non-participating group at C-2 and secondly, the β-imidate has to be used. It follows that reaction with inversion at C-1 would lead to the α-glycosylation product.

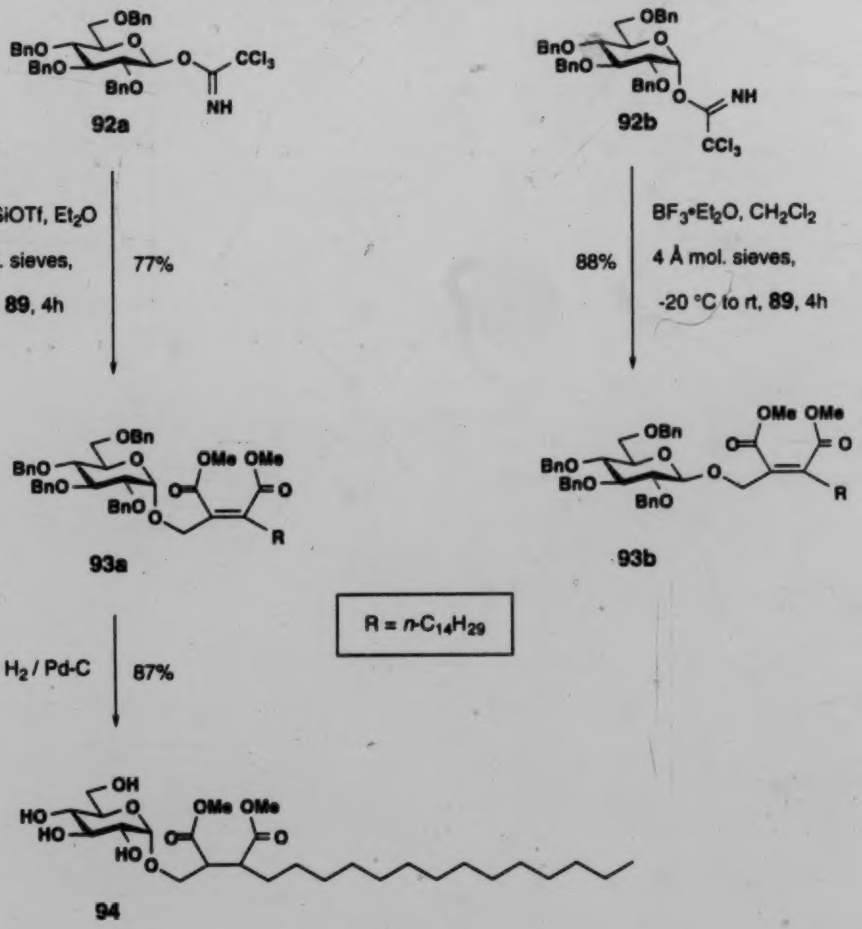
Therefore, for our synthetic plan, we sought imidate **92a** which fulfills the above-mentioned requirements. However, imidate **92a** immediately raised a concern about the last steps of the target synthesis, specifically at the deprotection stage. We were concerned about the risk that the double bond would be destroyed during the removal of the benzyl groups, especially in light of the results shown above for the deprotection of compound **87b**. Nonetheless, we were encouraged by the numerous methods that exist for the removal of benzyl groups, and the assumption that these sugar protecting benzyl groups should be more labile than the relatively stable tetrasubstituted double bond. Furthermore, we were attracted to imidate **92a** because of its ease of preparation from commercially available materials, and the fact that it is a stable yet reactive glycosylating agent.[243]

β-Imidate **92a** was prepared[244] from tetra-*O*-benzyl glucose **80** and trichloroactonitrile in the presence of K_2CO_3 as a base. The reaction proceeded with excellent yield (95 %) and good selectivity, giving **92a** as a 5:1 mixture with **92b**. The β-anomer **92a** was easily separated by chromatography from the α-anomer **92b**. In the presence of NaH as a base the reaction gave the α-imidate **92b** exclusively in 82% yield (Scheme 31).[245] For comparison reasons, especially at later stages in the synthesis, the α-imidate **92b** was used in an analogous reaction sequence.


OBn
O
BnO
BnO
BnO
OH
80
Cl3CCN
K2CO3, CH2Cl2, rt,
95% (1:5, α:β)
NaH, CH2Cl2, rt, 82%
OBn
O
BnO
BnO
BnO
O
CCl3
NH
92a
OBn
O
BnO
BnO
BnO
O
NH
CCl3
92b

Scheme 31.

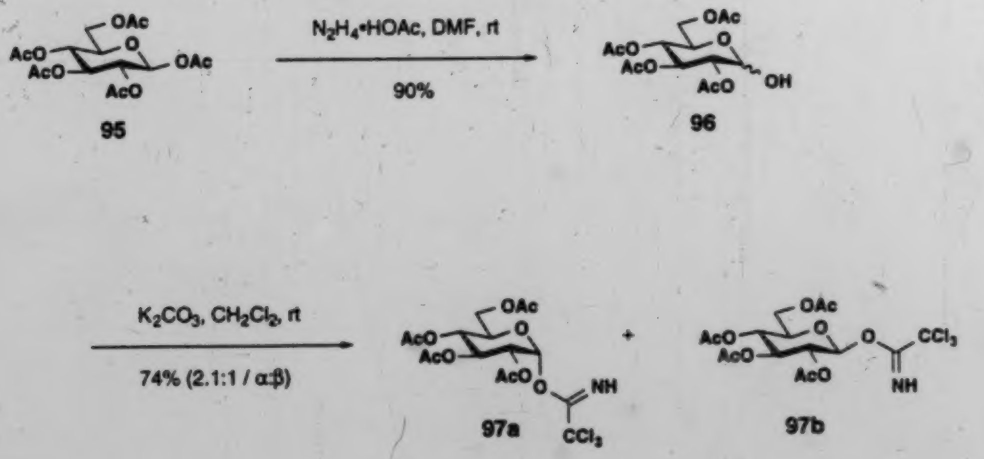
Having made both the glycosyl acceptor and donor, we then investigated the glycosylation reaction. Thus, when alcohol **89** and β-imidate **92a** in ether were reacted in the presence of molecular sieves and trimethylsilyl triflate as the promoter, the desired α-glycosylation product **93a** was obtained in 77 % yield. The analogous reaction with α-imidate **92b** in the presence of BF_3•Et_2O as the promoter and CH_2Cl_2 as the solvent gave the expected β-glycosylation product **93b** in 88 % isolated yield (Scheme 32). When hydrogenation was attempted on **93a**, unfortunately the fully hydrogenated


OBn
BnO
BnO
BnO
O
CCl₃
NH
92a
92b
(CH₃)₃SiOTf, Et₂O
4 Å mol. sieves,
-20 °C, 89, 4h
77%
88%
BF₃•Et₂O, CH₂Cl₂
4 Å mol. sieves,
-20 °C to rt, 89, 4h
OMe OMe
R
93a
93b
R = n-C₁₄H₂₉
H₂ / Pd-C
87%
OH
HO
94

Scheme 32.

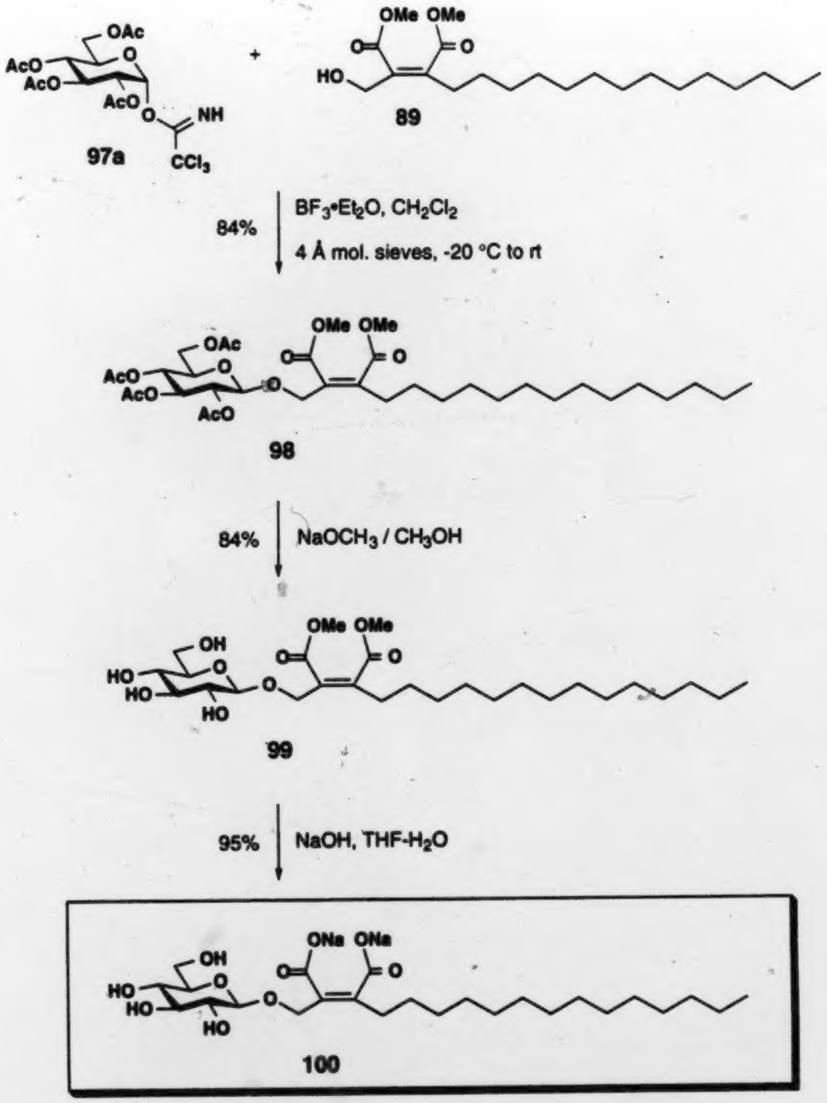
product **94** was obtained in 87 % yield. Despite several attempts using various hydrogenation conditions, the double bond was reduced in each case. The use of TMSI selectively removed the benzyl groups, but as expected it also caused cleavage of the glycosyl bond. Because this approach to the α-glycosylation product **93a** is simple and efficient, studies are still underway to develop a selective deprotection protocol.

In the meantime a similar approach, using a differently protected imidate, was investigated. The synthetic sequence employed the *O*-acetyl protected imidate **97a** which was prepared as shown in Scheme 33. Thus, selective deprotection of the anomeric hydroxyl in **95** using hydrazine acetate in DMF gave **96** mainly as the α-anomer. Imidate formation as before gave the known imidates **97a**[244] and **97b**.[245]


OAc
AcO
AcO
O
OAc
AcO
95
N₂H₄•HOAc, DMF, rt
90%
OAc
AcO
AcO
O
AcO
OH
96
K₂CO₃, CH₂Cl₂, rt
74% (2.1:1 / α:β)
OAc
AcO
AcO
O
AcO
O
NH
CCl₃
97a
+
OAc
AcO
AcO
O
AcO
O
CCl₃
NH
97b

Scheme 33.

The glycosylation reaction was then done, using alcohol **89** and imidate **97a** in CH_2Cl_2 in the presence of $BF_3{\cdot}Et_2O$, to give the coupled product **98** with the expected β-stereochemistery in 84 % yield. Removal of the acetate groups with methanolic sodium methoxide gave diester **99** which was hydrolyzed by sodium hydroxide to give the desired product **100** in 71 % overall yield from **89** (Scheme 34).


OAc
AcO
AcO
AcO
O
NH
CCl3
97a
+
OMe OMe
O
O
HO
89
84%
BF3•Et2O, CH2Cl2
4 Å mol. sieves, -20 °C to rt
98
84%
NaOCH3 / CH3OH
OH
HO
99
95%
NaOH, THF-H2O
ONa ONa
100

Scheme 34.

In summary, the synthesis of the type **B** target with β-stereochemistry has been achieved. Studies are in progress to complete the synthesis of the α-anomer.

3 Synthetic Studies on the Type C Targets.

The previous target molecules were designed to mimic the active monosaccharide portion of natural moenomycin A antibiotics shown to block transglycosylase. The peptidoglycan monomer (PGM), on the other hand, provides another structural motif for inhibitor design. The GlcNAc derivative was designed to examine the importance of the terminal GlcNAc unit in PGM and to model the synthesis of the disaccharide target derivative. The disaccharide portion of the target is the same as that of the transglycosylase substrate, PGM, without the peptide chain. The pyrophosphate group of PGM was replaced by the maleic acid moiety as a stable mimic (Figure 40).

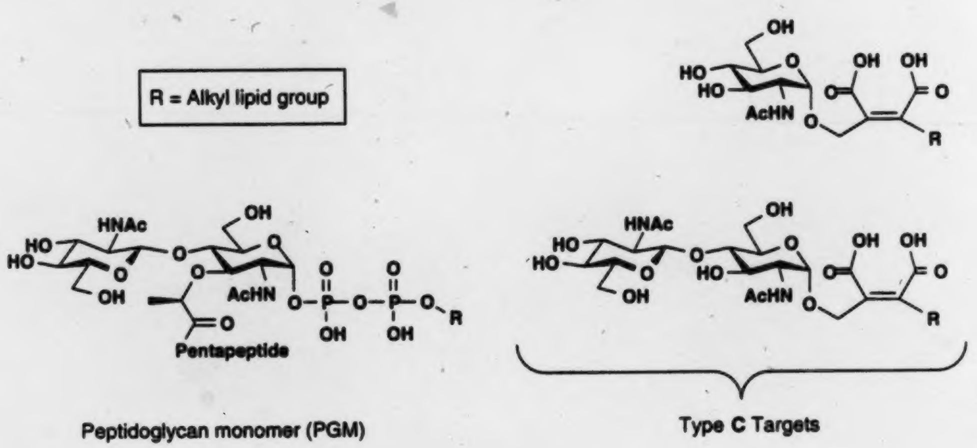
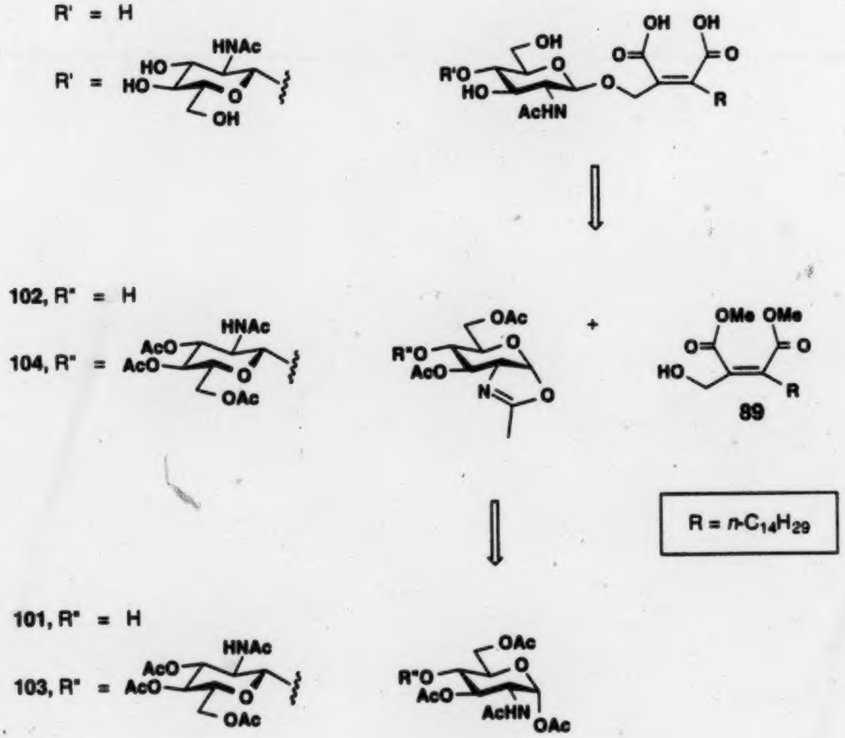


Figure 40.

In the following sections, we describe some of the synthetic studies toward the β-anomers of Type C targets. Even though the original design targeted the α-anomers (α at the glycosidic linkage between the sugar and the lipid moieties) since this is the stereochemistry that is present in the natural substrate (PGM), the β-anomers were studied first because they were envisaged to be easily accessible given the building blocks that we already had.

3.1 The Oxazoline Method.

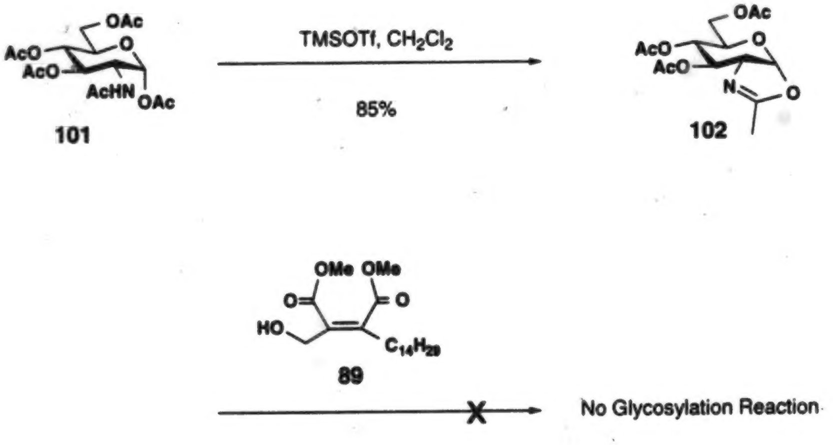
Considering the studies presented above, we thought that a convergent approach should also be applicable for the synthesis of the type **C** targets. The presence of the acetamido group at the C-2 position of both the mono- and disaccharide target molecules immediately suggested the oxazoline approach[246] as an appropriate synthetic strategy (Figure 41). Thus, activation of the oxazoline **102** or **104** followed by nucleophilic attack at the glycosidic center by alcohol **89** would result in ring opening with inversion of configuration at C-1 to give the 1,2-*trans*-glycoside. The main advantage of the oxazoline approach is that the required C-2 acetamido group is already in place.



R' = H
R' = HO HO HNAc O OH
OH OH
R'O OH O HO AcHN O O O R
102, R" = H
104, R" = AcO AcO HNAc O OAc
R"O OAc O AcO N O
+
OMe OMe
O O HO R
89
R = n-C14H29
101, R" = H
103, R" = AcO AcO HNAc O OAc
R"O OAc O AcO AcHN OAc

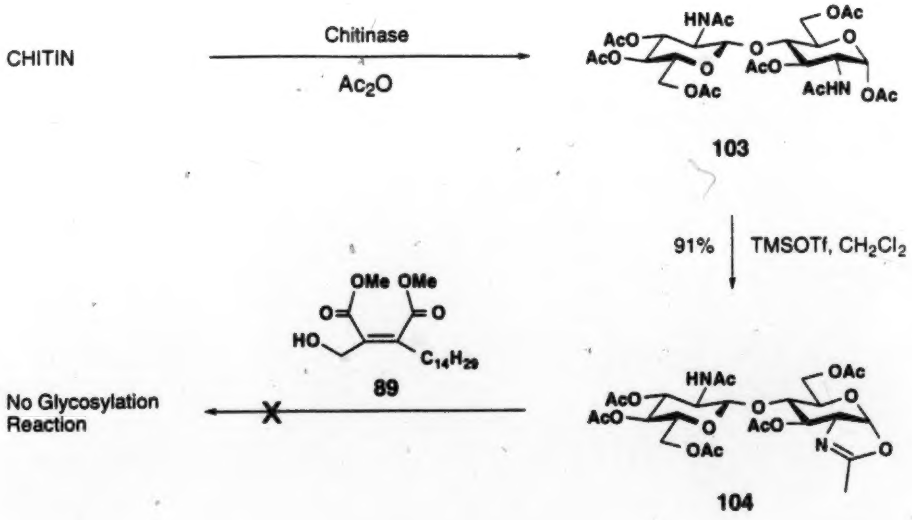
Figure 41.

The literature procedure was followed for the preparation of oxazoline **102** (Scheme 35).[247] Treatment of the pentaacetate derivative **101** with trimethylsilyl trifluoromethanesulfonate (TMSOTf) afforded the oxazoline **102**. Attempted glycosylation of alcohol **89** with oxazoline **102** in the presence of TMSOTf[248] did not lead to any of the desired product. The reaction was tried with different conditions, but with no success.


OAc
AcO
AcO
O
AcHN
OAc
101
TMSOTf, CH_2Cl_2
85%
OAc
AcO
AcO
O
N
O
102
OMe OMe
O
O
HO
$C_{14}H_{29}$
89
No Glycosylation Reaction

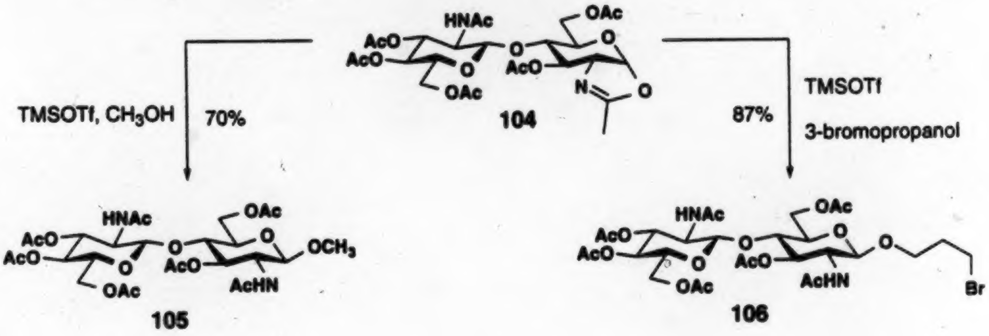
Scheme 35.

Similarly, oxazoline **104** was prepared as shown in Scheme 36. Enzymatic degradation of colloidal chitin using commercially available chitinase, followed by acetylation[249,250] gave the peracetylated chitobiose **103**.[251] Treatment of **103** with TMSOTf as described by Kuzuhara[252] gave oxazoline **104** in 91 % yield. Attempted glycosylation of **104** with alcohol **89** also failed and starting materials were recovered.


CHITIN
Chitinase
Ac2O
103
91%
TMSOTf, CH2Cl2
89
No Glycosylation Reaction
104

Scheme 36.

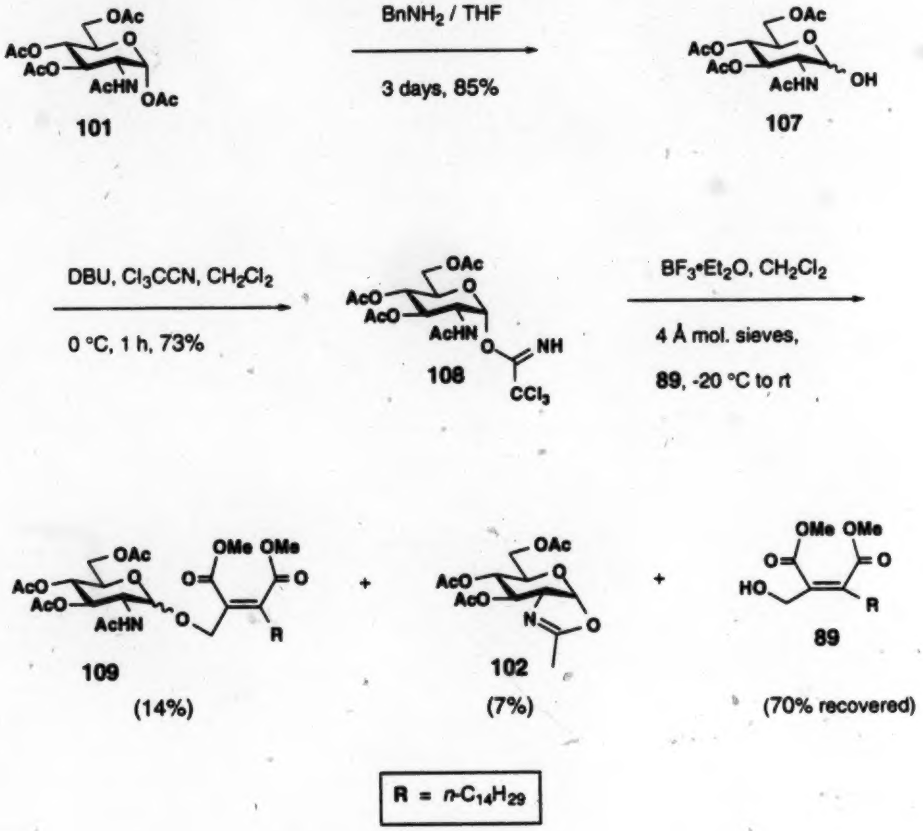
The above studies demonstrated the low reactivity of these oxazolines. In order to gauge the relative reactivity of alcohol **89**, oxazoline **104** was reacted with two primary alcohols, namely methanol and 3-bromopropanol. In both cases, the glycosylation products **105** and **106** respectively, were obtained (Scheme 37) suggesting perhaps that alcohol **89** may not be as reactive as expected.


104
TMSOTf, CH3OH
70%
87%
TMSOTf
3-bromopropanol
105
106

Scheme 37.

3.2 The Imidate Method.

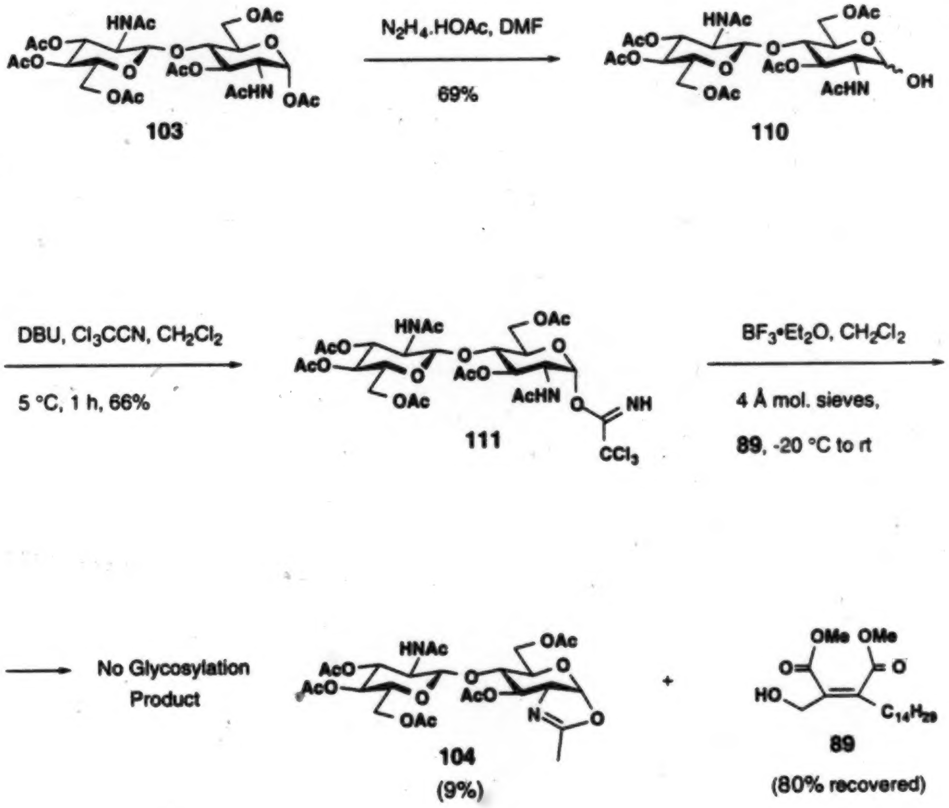
Having had relatively good success with the imidate approach for the synthesis of type **B** targets, we returned to this method in an attempt to solve the glycosylation problem encountered with oxazolines. Thus, selective anomeric deprotection of **101** gave derivative **107** which under standard imidate forming conditions using DBU as the base, provided the α-imidate **108** in 62 % yield over two steps. When the glycosylation reaction was tried on **108** using alcohol **89**, glycosylation proceeded sluggishly, giving derivative **109** as a mixture of anomers in a low yield (Scheme 38).



101
BnNH2 / THF
3 days, 85%
107
DBU, Cl3CCN, CH2Cl2
0 °C, 1 h, 73%
108
BF3•Et2O, CH2Cl2
4 Å mol. sieves,
89, -20 °C to rt
109
(14%)
102
(7%)
89
(70% recovered)
R = n-C14H29

Scheme 38.

The chitobiose imidate was also prepared as shown in Scheme 39. Deprotection of the anomeric hydroxyl of the peracetylated chitobiose **103** with hydrazine acetate gave derivative **110**. Reaction with trichloroacetonitrile in the presence of DBU at 5 °C gave imidate **111** in 66 % yield. Unfortunately, attempted glycosylation of imidate **111** with alcohol **89** failed to give any coupled product. Alcohol **89** was recovered unchanged along with 9 % of oxazoline **104** (Scheme 39).


103
N$_2$H$_4$.HOAc, DMF
69%
110
DBU, Cl$_3$CCN, CH$_2$Cl$_2$
5 °C, 1 h, 66%
111
BF$_3$•Et$_2$O, CH$_2$Cl$_2$
4 Å mol. sieves,
89, -20 °C to rt
No Glycosylation Product
104
(9%)
+
89
(80% recovered)

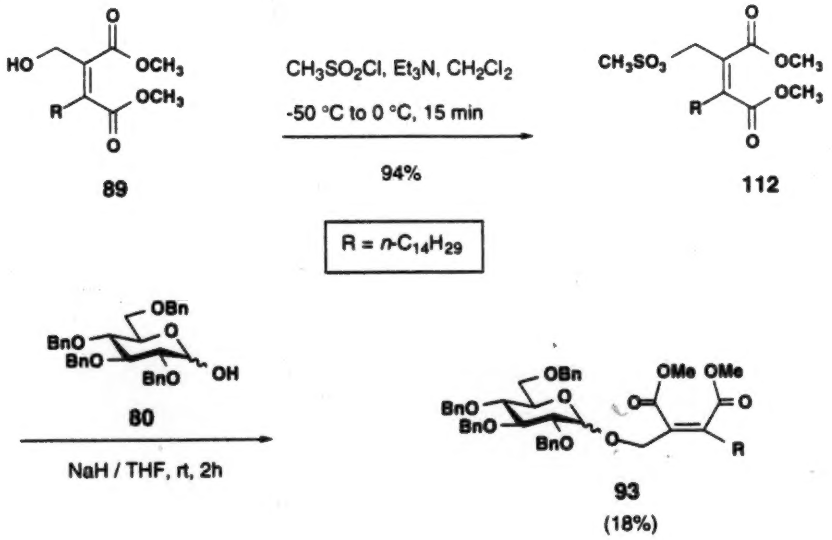
Scheme 39.

4 *O*-Glycosides *via* the Direct Anomeric *O*-Alkylation Method.

The direct anomeric *O*-alkylation of carbohydrates with simple alkylating agents (eg. methyl iodide and dimethyl sulfate) has long been known.[242] Of special interest is the application of this seemingly simple method to glycoside and saccharide synthesis. Schmidt and co-workers have shown that the direct anomeric *O*-alkylation method is very efficient in the alkylation of sugars with primary triflates. They used this method for the synthesis of various glycosides and disaccharides.[253] In fact in one example where the classical Koenigs-Knorr method failed to give the desired glycosylation product, a solution to the problem was provided by the direct anomeric *O*-alkylation method.[254]

In view of these encouraging results, we envisaged that this method may provide the solution to the problems we found during some of the *O*-glycosylation reactions presented above. Therefore, we had to convert alcohol **89** to an active electrophile then model its reaction with sugar *O*-1 alkoxides. Thus, the mesylate derivative **112** was prepared by reacting alcohol **89** with methanesulfonyl chloride in the presence of triethylamine. The anomeric *O*-alkylation of tetrabenzyl glucose **80** with mesylate **112** afforded the desired glycoside **93** as a mixture of anomers in 18 % yield (Scheme 40).

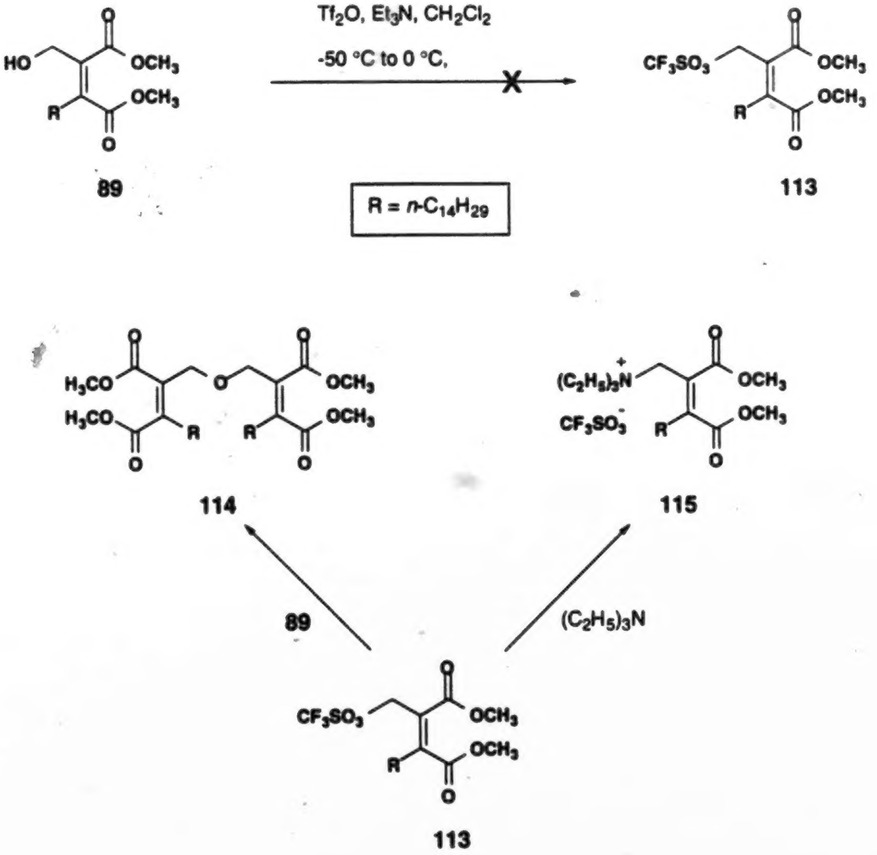
Although this method gave inferior results to those obtained by the imidate methodology presented above (Scheme 32), it showed that glycosides of this type may be rapidly accessible in a simple operation, provided that the right reaction conditions are used.



Scheme 40.

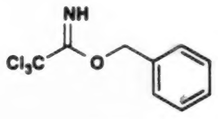
Based on literature precedent, primary triflates are the best electrophiles in these *O*-alkylation reactions. Hence, we attempted the preparation of triflate **113** from alcohol **89** (Scheme 41). In the reaction of alcohol **89** with triflic anhydride in the presence of triethylamine none of the desired allylic triflate **113** was isolated and instead the dimer **114** and the salt **115** were the major products, along with recovered alcohol **89**. ^{1}H NMR spectroscopy on the crude material showed that the main product was salt **115**. Dimer **114** was obtained in 13 % yield after column chromatography and it was also isolated in 10 % yield during attempted preparation of **113** using pyridine as the base.

It seems very likely that the desired triflate **113** was formed initially, but because of its reactivity it was attacked by alcohol **89**, and to a larger extent by triethylamine, to give **114** and **115** respectively (Scheme 41). Thus, triflate isolation requires modification of the reactants or reaction conditions to avoid displacement by triethylamine or pyridine. One modification to solve the problem could be to use a hindered non-nucleophilic base such as 2,6-di-*tert*-butyl-4-methylpyridine.[255] A second modification could be to the triflate at low temperature (-78 °C) in the presence of the desired nucleophile. These ideas are currently being investigated to establish optimal conditions for the isolation of triflate **113** and/or for its *in situ* alkylation with the sugar *O*-1 alkoxides.

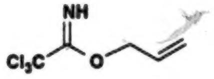

Tf₂O, Et₃N, CH₂Cl₂
-50 °C to 0 °C,
HO
OCH₃
R
CF₃SO₃
89
113
R = n-C₁₄H₂₉
H₃CO
(C₂H₅)₃N⁺
CF₃SO₃⁻
114
115
89
(C₂H₅)₃N
113

Scheme 41.

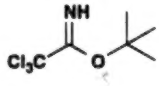
In 1985 Bundle and co-workers reported that benzyl and allyl trichloroacetimidates **116** and **117** are convenient reagents for the *O*-alkylation of hydroxyl groups of carbohydrate derivatives.[256] Because this novel method allows for *O*-alkylation under mildly acidic conditions which are compatible with a variety of functional groups, these reagents and others such as **118** rapidly found their way into use for important synthetic transformations such as the protection of alcohols[257] and acids.[258]



116

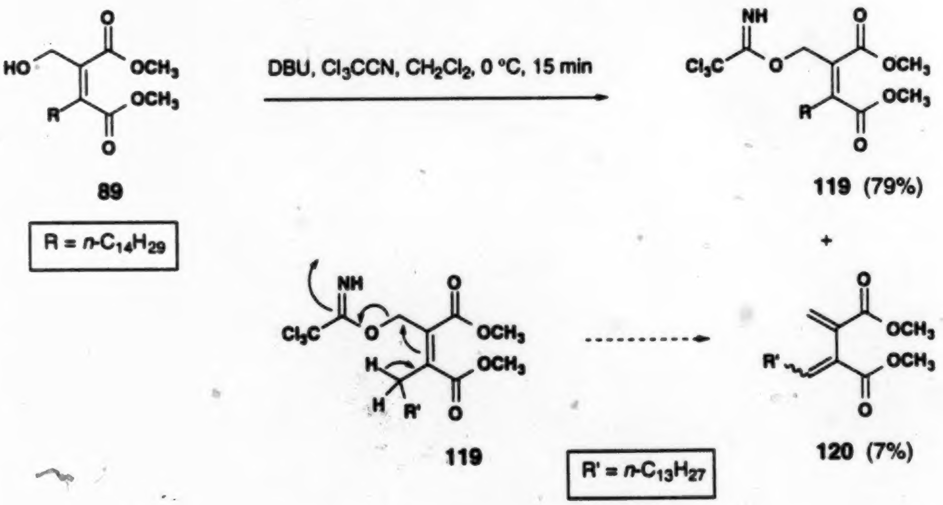


117



118

These studies encouraged us to activate alcohol **89** by converting it to the corresponding allylic trichloroacetimidate **119** and then investigate its behavior during the anomeric *O*-alkylation method. Thus, treatment of **89** with trichloroacetonitrile in the presence of DBU for 15 min at 0 °C gave the desired imidate **119** in 79 % yield. A small amount (7 %) of diene **120** was also obtained, which presumably was formed *via* base-catalyzed elimination of the trichloroacetamide group (Scheme 42). Investigations on the use of **119** in the direct anomeric *O*-alkylation method are underway.


HO
O
OCH_3
OCH_3
R
O
89
$R = n\text{-}C_{14}H_{29}$
DBU, Cl_3CCN, CH_2Cl_2, 0 °C, 15 min
NH
Cl_3C
O
OCH_3
OCH_3
R
O
119 (79%)
+
NH
Cl_3C
O
O
OCH_3
OCH_3
H
H
R'
O
119
$R' = n\text{-}C_{13}H_{27}$
O
OCH_3
OCH_3
R'
O
120 (7%)

Scheme 42.

CHAPTER 4 Experimental Procedures

General Methods.

All processes involving air or moisture sensitive reagents were performed under an atmosphere of dry argon using oven-dried glassware. Reagents and solvents were reagent grade and used as supplied unless otherwise stated. Solvents for anhydrous reactions were dried according to Perrin *et al.*[259] Tetrahydrofuran (THF), diethyl ether, 1,2-dimethoxyethane (DME), benzene and toluene were distilled from sodium and benzophenone under an argon atmosphere. Acetonitrile, dichloromethane, carbon tetrachloride, hexamethyldisilazane (HMDS), triethylamine and pyridine were distilled from calcium hydride. *N,N*-Dimethylformamide (DMF) was stirred with BaO (18 h), was decanted and was distilled at reduced pressure. Methanol and ethanol were distilled over magnesium turnings and a catalytic amount of iodine. Dimethyl sulfoxide (DMSO) was distilled from calcium hydride and was stored over CaH_2. Water was obtained from a Milli-Q reagent water system (Millipore Corp.; Milford, MA). "Brine" refers to a saturated aqueous solution of NaCl. Unless otherwise specified, solutions of NH_4Cl, $NaHCO_3$, KOH, and NaOH refer to aqueous solutions. Solvent evaporation was performed under reduced pressure below 40 °C using a Büchi rotary evaporator, followed by evacuation (< 0.1 torr) to constant sample weight.

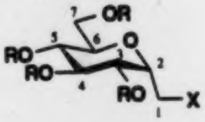
All reagents employed were of American Chemical Society (ACS) grade or finer and were used without further purification unless otherwise stated. Copper (I) bromide-dimethyl sulfide complex ($CuBr{\bullet}Me_2S$) was either used fresh from commercial sources or was prepared according to the method of House *et al.*[260] Recrystallization[261] of $CuBr{\bullet}Me_2S$ which came from old bottles was required to obtain a good quality reagent. Copper iodide (CuI) was either purchased from Aldrich Chemical Co. and was of high purity (> 99.9999 %) or purified according to the method of Kauffman *et al.*[262] Dimethyl azodicarboxylate was distilled at reduced pressure (safety shield) before use (72-73 °C / 2 mm Hg). Hexamethylphosphoric triamide (HMPA) was dried by stirring with calcium hydride under argon for 36 h, followed by distillation at reduced pressure and was stored over molecular sieves. Organometallic solutions, purchased from Aldrich, were periodically titrated against menthol/phenanthroline.

Reactions and fractions from column chromatography were monitored and analyzed by thin-layer chromatography (TLC) using glass plates with a UV fluorescent indicator (normal silica, Merck 60 F_{254}; reverse phase, Merck RP-8 and RP-18 F_{254}). One or more of the following methods were used for visualization: UV absorption by fluorescence quenching; iodine staining; phosphomolybdic acid/ceric sulfate/sulfuric acid (10 g : 1.25 g : 8 % 250 mL) spray; 50 % sulfuric acid spray; and 0.1 % $KMnO_4$ spray. Flash column chromatography was performed according to the method of Still *et al*[263] using 230-400 mesh silica (Merck, silica gel). Ion exchange resins AG1-X8 (Cl^- form, 100-200 mesh) and AG50W-X8 (H^+ form, 50-100 mesh) were purchased from Bio-Rad.

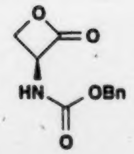
Reverse phase medium pressure liquid chromatography (MPLC) was performed on a Merck Lobar LiChroprep RP-8 column (40-63 µm), size A (24 x 1 cm) using solvents which were previously degassed under vacuum. High pressure liquid chromatography (HPLC) was performed on either a Beckman System Gold instrument equipped with a model 166 variable wavelength UV detector and an Altex 210A injector with a 100 µL sample loop, or on a Rainin instrument equipped with a Rainin UV-1 detector set at 250 nm and an injector fitted with a 5 mL sample loop. The columns were Waters Nova-Pak cartridges (reverse phase 8NVC18 4 µm C_{18} column) and Waters Resolve cartidges (reverse phase PrePak C_{18} column). All HPLC solvents were prepared fresh daily and filtered with a Millipore filtration system under vacuum before use.

Melting points were determined on a Thomas-Hoover or Büchi oil immersion apparatus using open capillary tubes and are uncorrected. Optical rotations were measured on a Perkin Elmer 241 polarimeter with a microcell (10.0 cm path length, 0.9 mL) at ambient temperature. All specific rotations reported were measured at the sodium D line and were referenced against air. Infrared spectra (IR) were recorded on Nicolet 7199 or 20 SX FT-IR spectrometers. Cast refers to the evaporation of a solution on a NaCl plate. Mass spectra (MS) were recorded on Kratos AEI MS-50 (high resolution mass spectrometry (HRMS), electron impact ionization (EI)), MS-12 (chemical ionization (CI), NH_3), and MS-9 (fast atom bombardment (FAB), argon) instruments. Cleland matrix used in FAB refers to a 5:1 mixture of dithiothreitol and dithioerythritol. Microanalyses were obtained on Perkin Elmer 240 or Carlo Erba 1180 elemental analyzers.

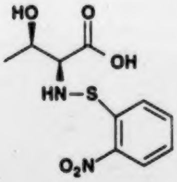
Nuclear magnetic resonance (NMR) spectra were obtained on Bruker WH-200, AM-300, WM-360, WH-400, or Varian 500 instruments. ^{1}H NMR chemical shifts are reported in parts per million (ppm) downfield relative to tetramethylsilane (TMS) using the solvent resonance as the reference: $CDCl_3$ δ 7.26, CD_2Cl_2 δ 5.32, D_2O δ 4.72, CD_3OD δ 3.30, and $(CD_3)_2NCOD$ δ 2.76. ^{13}C shifts are reported relative to $CDCl_3$ δ 77.0, CD_2Cl_2 δ 53.8, CD_3OD δ 49.0, and $(CD_3)_2CO$ δ 29.8. Selective homonuclear decoupling, shift correlation spectroscopy (COSY), attached proton test (APT), and ^{1}H-^{13}C correlation experiments were occasionally used for signal assignments. ^{1}H NMR data are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; qn, quintet and m, multiplet), number of protons, coupling constant (*J*) in Hertz (Hz) and assignment. When appropriate, the multiplicity is proceeded by br, indicating that the signal was broad. For ^{1}H NMR assignment only, protons of the (D-glucopyranosyl)methyl group (C-glycosides) present in the compounds described are numbered as indicated below. The methylene protons are defined as Ha and Hb.



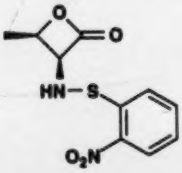
All literature compounds had IR, ^{1}H NMR, and mass spectra consistent with the reported data.



N-(Benzyloxycarbonyl)-L-serine β-lactone (7). The literature[71] procedure was modified. To a stirred solution of dried Ph_3P (21.1 g, 80.4 mmol) in dry THF (400 mL) at -78 °C was added distilled dimethyl azodicarboxylate (11.7 g, 80.0 mmol, 8.85 mL) dropwise over 40 min. The resulting orange solution was stirred at -75 °C for 10 min, at which point a milky white slurry was obtained. A solution of *N*-(benzyloxycarbonyl)-L-serine (19.2 g, 80.0 mmol) in dry THF (120 mL) was added dropwise over 20 min to the well stirred slurry at -75 °C. After completion of the addition, the mixture was stirred for 20 min at -75 °C and then allowed to warm to 20 °C and stirred for 2.5 h. The solvent was removed *in vacuo* at 35 °C and the residue obtained was purified by flash chromatography on silica gel (hexane-EtOAc, 5.5:4.5) to afford 7 (7.9 g, 45 %) as a white solid. An analytical sample was recrystalized from EtOAc / hexane (20 °C to -20 °C) to give white needles: mp 130-132 °C (lit.[71] m p 133-134 ° C); IR (CH_2Cl_2 cast) 3360, 1843, 1828, 1685, 1530, 1271 cm^{-1}; ^1H NMR (CD_2Cl_2, 400 MHz) δ 7.35 (s, 5 H, Ar$\underline{H}$), 5.70-5.62 (br s, 1 H, N$\underline{H}$), 5.11 (s, 2 H, OC$\underline{H}_2$Ph), 5.02 (m, 1H, C$\underline{H}_\alpha$), 4.42 (m, 2 H, -C$\underline{H}_2$-O); ^{13}C NMR (CD_2Cl_2, 100 MHz) δ 169.4 (C=O lactone), 155.9 (C=O urethane), 136.2 (Ar$\underline{C}$), 129.1, 128.9 and 128.7 (Ar$\underline{C}$H), 68.0 and 66.4 (Ph$\underline{C}H_2$O and -CH_2O-), 60.1 ($\underline{C}$H); HRMS (EI) Calcd for $C_{11}H_{11}NO_4$ 221.0688, found 221.0692. Anal. Calcd for $C_{11}H_{11}NO_4$: C, 59.73; H, 5.01; N, 6.33. Found: C, 59.93; H, 5.02; N, 6.36.

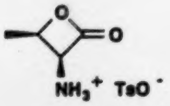


***N*-[(*o*-Nitrophenyl)sulfenyl]-L-threonine (10).** The literature[94] procedure was modified. L-Threonine (4.47 g, 37.5 mmol) was dissolved in dioxane (46 mL) and 2 N NaOH (19 mL). To the vigorously stirred solution was added *o*-nitrophenylsulfenylchloride (8.05 g, 42.5 mmol) in eight equal portions over 35 min, while 2 N NaOH (23 mL) was added dropwise to maintain a pH of 9.0. After stirring for 30 min at 20 °C, the reaction mixture was diluted with water (150 mL) and extracted with EtOAc (3 x 80 mL). The aqueous solution was acidified to pH 2.5 with 10 % $KHSO_4$ and was then immediately extracted with EtOAc (3 x 100 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give a yellow solid. Recrystallization from acetone / hexane afforded the known **10** (7.90 g, 77 %) as yellow crystals: mp 143-145 °C (lit.[94] mp 145-148 °C); IR (KBr) 3410 , 3300-2400 (br), 1742, 1509, 1330, 737 cm^{-1}; ^1H NMR (CD_3OD, 200 MHz) δ 8.25 (m, 2 H, Ar$\underline{H}$), 7.68 (m, 1 H, Ar$\underline{H}$), 7.30 (m, 1 H, Ar$\underline{H}$), 4.20 (m, 1 H, C$\underline{H}_\beta$), 3.38 (d, 1 H, J = 4.4 Hz, C$\underline{H}_\alpha$), 1.40 (d, 3 H, J = 6.2 Hz, C$\underline{H}_3$); HRMS (EI) Calcd for $C_{10}H_{12}N_2O_5S$ 272.0469, found 272.0466. Anal. Calcd for $C_{10}H_{12}N_2O_5S$: C, 44.11; H, 4.44; N, 10.29; S, 11.77. Found: C, 44.19; H, 4.48; N, 10.09; S, 11.91.

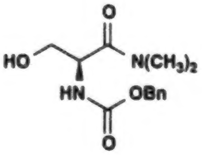


(3*S*,4*R*)-3-[[(*O*-Nitrophenyl)sulfenyl]amino]-4-methyl-2-oxetanone (11). The literature[84] procedure was modified. To a solution of **10** (1.00 g, 3.9 mmol) in pyridine (15 mL) at -43 °C was added a solution of 4-bromobenzenesulfonyl chloride (2.00 g, 8.00

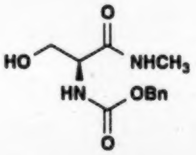
mmol) in dry pyridine (15 mL) at 0 °C dropwise over 10 min. The mixture was stirred at -43 °C for 1 h, was allowed to warm to 0 °C over a period of 3 h, and then poured into ice water (50 mL). This mixture was acidified with concentrated HCl to pH 2 and was immediately extracted with EtOAc (5 x 50 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give an orange oil. Purification by flash chromatography (hexane-EtOAc, 6:4) gave **11** (600 mg, 60 %) as a yellow solid: mp 125-127 °C (lit.[84] mp 134-135 °C); IR ($CHCl_3$ cast) 1814, 1509, 1338, 734 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.34 (m, 1 H, ArH), 8.10 (m, 1 H, ArH) 7.80 (m, 1 H, ArH), 7.39 (m, 1 H, ArH), 4.92 (q, 1 H, *J* = 6.3 Hz, CH$_\beta$), 4.74 (dd, 1 H, *J* = 8.2, 6.3 Hz, CH$_\alpha$), 3.52 (d, 1 H, *J* = 8.2 Hz, NH), 1.6 (d, 3 H, *J* = 6.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 169.8 (C=O), 134.7 and 127.4 (ArC), 126.5, 126.2, 125.6 and 124.1 (ArCH), 75.9 and 70.9 (CH), 15.4 (CH_3); HRMS (EI) Calcd for $C_{10}H_{10}N_2O_4S$ 254.0361, found 254.0359. Anal. Calcd for $C_{10}H_{10}N_2O_4S$: C, 47.24; H, 3.96; N, 11.02; S, 12.61. Found: C, 46.99; H, 3.93; N, 10.81; S, 12.51.



(3*S*,4*R*)-3-Amino-4-methyl-2-oxetanone *p*-toluenesulfonate salt (12). The literature[84] procedure was modified. To a stirred suspension of **11** (100 mg, 0.40 mmol) in CH_2Cl_2 (1 mL) under argon was added anhydrous *p*-toluenesulfonic acid (74 mg, 0.43 mmol), followed by *p*-thiocresol (100 mg, 0.800 mmol). After stirring the mixture at 20 °C for 6 h, the solvent was evaporated and the resulting yellow solid was triturated with diethyl ether until it was colorless. Recrystallization from EtOAc / hexane afforded **12** (79 mg, 72 %) as a white solid: mp 126-127 °C (dec.) (lit.[84] mp 120 °C); IR (KBr) 3150, 1840, 1496, 1204 cm^{-1}; ^{1}H NMR (DMF-d_7, 400 MHz) δ 7.61 (d, 2 H, *J* = 7.3 Hz, ArH), 7.12 (d, 2 H, *J* = 7.3 Hz, ArH), 5.52 (d, 1 H, *J* = 7.4 Hz, $CHNH_3^+$), 5.15 (q, 1 H, *J* = 7.0 Hz, $CHCH_3$), 2.26 (s, 3 H, $ArCH_3$); MS (FAB, glycerol) *m/z* (relative intensity) 274 (MH^+, 12 %). Anal. Calcd for $C_{11}H_{15}NO_5S$: C, 48.35; H, 5.49; N, 5.12; S, 11.72. Found: C, 48.25; H, 5.36; N, 5.01; S, 11.39.

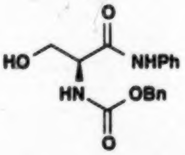


***N*-(Benzyloxycarbonyl)-L-serine *N,N*-dimethylamide (16).** To a stirred suspension of dimethylamine hydrochloride (81.5 mg, 1.00 mmol) in dry CH_2Cl_2 (3 mL) was added dimethylaluminum chloride (1.00 mL of a 1.0 M solution in hexanes, 1.00 mmol) at 0 °C. After the addition was complete, the suspension was allowed to warm to room temperature and was stirred for an additional 5 min, during which time it became clear and colorless. The solution was then cooled to 0 °C and *N*-Cbz-L-serine β-lactone **7** (111 mg, 0.500 mmol) in dry CH_2Cl_2 (4 mL) was added. The mixture was allowed to warm to room temperature and stirring was continued until β-lactone consumption was complete (2 h), as shown by tlc. The mixture was then quenched at 0 °C by the addition of 0.2 M HCl (10 mL, pre-cooled to 5 °C). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 15 mL). The combined organic extracts were washed with H_2O (30 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give the crude product. Recrystallization (ethyl acetate / hexane) afforded **16** (110 mg, 84 %) as white solid: mp 124-125 °C; IR (CH_2Cl_2 cast) 3600-3000 (br), 1717, 1635, 1530 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.40-7.20 (m, 5 H, Ar$\underline{H}$), 5.08 (s, 2 H, PhC$\underline{H}_2$O), 4.75 (t, 1 H, J = 6.0 Hz, -C$\underline{H}_\alpha$), 3.70 (dd, 1 H, J = 11.1, 5.6 Hz, -CH$\underline{H}$-OH), 3.64 (dd, 1 H , J = 11.1, 5.6 Hz, C$\underline{H}$H-OH), 3.30, and 2.95 (2s, 6 H, N(C$\underline{H}_3$)$_2$); ^{13}C NMR (CD_3COCD_3, 75 MHz) δ 171.0 ($\underline{C}$=O amide), 156.7 ($\underline{C}$=O urethane), 138.2 (Ar$\underline{C}$), 129.2 and 128.6 (Ar$\underline{C}$H), 66.8 and 63.7 ($\underline{C}H_2$OH and Ph$\underline{C}H_2$O), 53.5 ($\underline{C}H_\alpha$), 37.2 and 35.6 (N($\underline{C}H_3$)$_2$); MS (CI, NH_3) m/z (relative intensity) 267 (MH^+, 100 %); HRMS (EI) Calcd for $C_{13}H_{18}O_4N_2$ 266.1267, found 266.1266. Anal. Calcd for $C_{13}H_{18}O_4N_2$: C, 58.63; H, 6.81; N, 10.52. Found: C, 58.54; H, 6.68; N, 10.21.



N-(Benzyloxycarbonyl)-L-serine N-methylamide (17). To a stirred suspension of methylamine hydrochloride (135 mg, 2.00 mmol) in dry CH_2Cl_2 (5mL) was added trimethylaluminium (1.00 mL of a 2.0 M solution in hexane, 2.00 mmol) at 0 °C. After the addition was complete, the cloudy suspension was allowed to warm to room temperature and was stirred for an additional 5 min, during which time it became clear and colorless. It was then cooled to 0 °C, and *N*-Cbz-L-serine-β-lactone **7** (221 mg, 1.00 mmol) in dry CH_2Cl_2 (4 mL) was added. The milky mixture was allowed to warm to room temperature and stirring was continued until β-lactone consumption was complete (2 h), as shown by tlc. The mixture was then cooled to 0 °C and 0.2 M HCl (20 mL, pre-cooled to 5 °C) was slowly added (exothermic reaction) so as to keep the temperature below 5 °C. The resulting white suspension was warmed to 20 °C and stirred for 30 min. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 30 mL). The combined organic extracts were washed with H_2O (40 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give **17** (204 mg, 81%) as a white solid. For an analytically pure sample this material was recrystallized from ethyl acetate / hexane to give **17** as white crystals: mp 110-111 °C; IR (KBr) 3600-3000 (br), 1686, 1646, 1539 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.38 (s, 5 H, ArH), 6.70-6.60 (br s, 1 H, CH_3NH), 5.95-5.85 (br d, 1 H, J = 8.3 Hz, urethane NH), 5.10 (s, 2 H, $-OCH_2Ph$), 4.30-4.05 (m, 2 H, CH_α and HOCHH), 3.62 (dd, 1 H, J = 13.2, 6.7 Hz, HOCHH), 2.80 (d, 3 H, J = 8.4 Hz, CH_3); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ 171.6 (C=O amide), 157.0 (C=O, urethane), 138.1 (ArC), 129.2 and 128.6 (5 ArCH), 66.9 and 63.5 (CH_2O and $PhCH_2O$), 57.7 (CH_α), 26.1

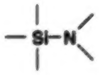
($\underline{C}H_3$-N); MS (FAB, cleland) *m/z* (relative intensity) 253.03 (MH^+, 100); HRMS (EI) Calcd for $C_{12}H_{16}N_2O_4$ 252.1110, found 252.1109. Anal Calcd for $C_{12}H_{16}N_2O_4$: C, 57.13; H, 6.39; N, 11.10. Found: C, 57.22; H, 6.38; N, 11.14.



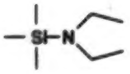
***N*-(Benzyloxycarbonyl)-L-serine *N*-phenylamide (18).** To a stirred solution of aniline (186 mg, 2.00 mmol) in dry CH_2Cl_2 (5 mL) was added dimethylaluminum chloride (2.00 mL, 1 M solution in hexane, 2.00 mmol) at 5 °C. After the addition was complete, the cloudy mixture was allowed to warm to room temperature and was stirred for a further 20 min. The reaction mixture was then cooled to 0 °C, and *N*-Cbz-L-serine β-lactone **7** (221 mg, 1.00 mmol) in dry CH_2Cl_2 (4 mL) was added. The clear solution was allowed to warm to room temperature and stirring was continued until β-lactone consumption was complete (3 h), as indicated by tlc. The solution was cooled to 0 °C and 0.2 M HCl (15 mL, pre-cooled to 4 °C) was slowly added (exothermic reaction) so as to keep the temperature below 5 °C. The resulting white suspension was then warmed to room temperature and stirred for 30 min. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 15 mL). The combined organic extracts were washed with H_2O (50 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give **18** (252 mg, 89 %) as a white solid (tlc, ethyl acetate-hexane, 6 : 4, R_f 0.38). For an analytically pure sample this material was recrystallized from ethyl acetate-hexane to afford **18** as a crystalline solid: mp 160-161 °C; IR (KBr) 3392, 3290, 1698, 1686, 1662, 1600, 1546 cm^{-1}; ^1H NMR (CD_3COCD_3, 400 MHz) δ 9.25 (s, 1 H, PhN$\underline{H}$), 7.66-7.63 (m, 2 H, Ar$\underline{H}$), 7.39-7.26 (m, 7 H, Ar$\underline{H}$), 7.08-7.04 (m, 1 H, Ar$\underline{H}$), 6.49-6.48 (br d, 1 H, J = 4.2 Hz, urethane N$\underline{H}$), 5.09 (s, 2 H, OC$\underline{H}_2$Ph), 4.39-4.34 (dt, 1 H, J = 8.0, 5.1 Hz, C$\underline{H}_\alpha$), 4.30 (br s, 1 H, $\underline{H}OCH_2$), 3.93 (dd, 1 H, J = 11.2, 5.4 Hz, HOC$\underline{H}$H), 3.85 (dd, 1 H, J = 11.2, 5.6

Hz, HOCH$\underline{H}$); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ 174.0 ($\underline{C}$=O amide), 159.8 ($\underline{C}$=O urethane), 139.8 and 138.0 (Ar$\underline{C}$), 129.5, 129.2, 128.7 and 124.5 (Ar$\underline{C}$H), 67.0 and 63.3 ($\underline{C}H_2OH$ and Ph$\underline{C}H_2O$), 58.5 ($\underline{C}H_\alpha$); HRMS (EI) Calcd for $C_{17}H_{18}N_2O_4$ 314.1266, found 314.1257. Anal. Calcd for $C_{17}H_{18}N_2O_4$: C, 64.96; H, 5.77; N, 8.91. Found: C, 65.30; H, 5.79; N, 8.98.

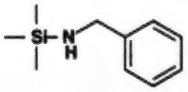
Compound **18** was also prepared in the same manner in 75 % and 92 % yields using trimethylaluminum and aluminum trichloride, respectively, as the Lewis acids.



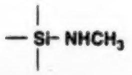
***N,N*-Dimethyl(trimethylsilyl)amine (19).** The literature procedure[264] was followed. To a solution of dimethylamine(22 mL, 0.5 mol) in dry ether (100 mL) at 0 °C was added trimethylchlorosilane (11 g, 0.10 mol) in ether (40 mL). After the addition was complete, the reaction mixture was allowed to warm to room temperature and stirring was continued for an additional 2h. After removal of the solvent, the remaining liquid was fractionally distilled to give**19** (8.2 g, 70 %) as a colorless liquid: bp 84-85 °C (760 Torr) (lit. bp 85-86 °C, 755 Torr); IR (neat film) 2958, 1253, 1058, 844 cm^{-1}; ^{1}H NMR (CD_2Cl_2, 360 MHz) δ 2.45 (s, 6 H, $(C\underline{H}_3)_2N$-), 0.05 (s, 9 H, $(C\underline{H}_3)_3Si$). Anal. Calcd for $C_5H_{15}NSi$: C, 51.21; H,12.89; N, 11.94. Found: C, 49.98, H, 12.71, N, 11.87.



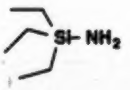
***N,N*-Diethyl(trimethylsilyl)amine (20).** The literature procedure[264] was followed. To a stirred solution of freshly distilled diethylamine (3.71 g, 50.0 mmol) in dry ether (25 mL) at -60 °C was added *n*-butyllithium (20 mL of a 2.5 M solution in hexane, 50.0 mmol). After the addition was complete, the reaction mixture was allowed to warm to 10 °C and stirring was continued for 20 min. The clear solution was then cooled to -50 °C and trimethylchlorosilane (5.42 g, 50.0 mmol) in dry ether (25 mL) was added dropwise over 10 min. The cloudy mixture was allowed to warm to room temperature, at which point a white suspension formed, and stirring was continued for 8 h. The solid was then removed by filtration and was washed with dry ether (4 x 30 mL). After removal of the solvent, the remaining pale yellow liquid was fractionally distilled to give **20** (5.2 g, 71 %) as a colorless liquid: bp 124-125 °C (755 Torr) (lit.[264] bp 125-126 °C, 760 Torr); IR (neat film) 2929, 1373, 1245, 834 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.78 (q, 4 H, *J* = 7.0 Hz, $(CH_3C\underline{H}_2)_2N$), 0.96 (t, 6 H, *J* = 7.1 Hz, $(C\underline{H}_3CH_2)_2N$), 0.02 (s, 9 H, $(C\underline{H}_3)_3Si$); HRMS (EI) Calcd for $C_7H_{19}NSi$ 145.1287, found 145.1295.



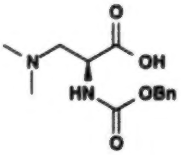
***N*-Benzyl(trimethylsilyl)amine (21).** The literature procedure[265] was followed. To a solution of freshly distilled benzylamine (31.9 g, 0.302 mol) in dry benzene (100 mL) at 0 °C was added trimethylchlorosilane (10.9 g, 0.102 mol) in dry benzene (20 mL) over 10 min with vigorous stirring. The thick, white suspension that immediately formed was stirred for 30 min at 5 °C, then allowed to warm to room temperature and stirred for an additional 3 h. The reaction mixture was then filtered under argon and the residue was washed with dry benzene (2 x 100 mL). After removal of the solvent (under argon) the remaining liquid was fractionally distilled to give **21** (11 g, 60 %) as a colorless liquid: bp 94-95 °C (0.8 Torr); IR (neat film) 3400, 2954, 1397, 1248, 870, 830 cm^{-1}; ^{1}H NMR (CD_2Cl_2, 200 MHz) δ 7.45-7.20 (m, 5 H, $ArC\underline{H}$), 4.05 (d, 2 H, *J* = 8.0 Hz, $C\underline{H}_2N$), 0.91 (br s, 1H, $N\underline{H}$), 0.16 (s, 9 H, $(C\underline{H}_3)_3Si$); ^{13}C NMR (CD_2Cl_2, 100 MHz) δ 147.1 ($Ar\underline{C}$), 131.4, 130.2, 129.5 ($Ar\underline{C}H$), 49.1 ($\underline{C}H_2N$), 3.0 ($(\underline{C}H_3)_3Si$); HRMS (EI) Calcd for $C_{10}H_{17}NSi$ 179.1130, found 179.1123. Anal. calcd for $C_{10}H_{17}NSi$: C, 66.97; H, 9.55; N, 7.81. Found: C, 67.03; H, 9.86; N, 7.82.



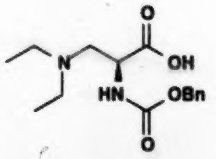
N-Methyl(trimethylsilyl)amine (22). The literature[266] method was followed. In a dry ice/acetone cooled 3-necked round bottomed-flask, fitted with a dropping funnel, a magnetic stirrer, a glass tubing for gas inlet and a gas condenser, was condensed about 16 mL (excess) of anhydrous methylamine. To this was added dry ether (50 mL) followed by a dropwise addition of a solution of trimethylchlorosilane (11 g, 0.10 mol) in dry ether (50 mL). A white suspension formed immediately and this accumulated as the addition proceeded. After the addition of the silane reagent was complete, the reaction mixture was allowed to warm to room temperature and stirring was continued for an additional 3 h, at which point most of the excess methylamine had already evaporated. The remaining methylamine was removed by warming the reaction flask to 35 °C. The thick white suspension was then extracted with dry ether (3 x 100 mL) and filtered. The ether was carefully removed and the remaining liquid was fractionally distilled to afford **22** (1.1 g, 21 %) as a colorless liquid: bp 69-71 °C (755 Torr) (lit.[266] bp 71 °C, 760 Torr); IR ($CDCl_3$ cast) 3327, 2912, 1251, 1064, 907, 838 cm^{-1}; ^1H NMR (CD_2Cl_2, 200 MHz) δ 2.45 (s, 3 H, $C\underline{H}_3$), 0.43-0.25 (br s, 1 H, $N\underline{H}$), 0.03 (s, 9 H, $(C\underline{H}_3)_3Si$); HRMS (EI) Calcd for $C_4H_{13}NSi$ 103.0817, found 103.0824.



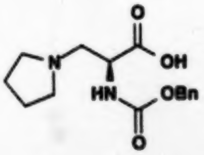
Triethylsilylamine (23). The literature[264] procedure was modified as follows: In a 250-mL three-necked round bottomed-flask, equipped with a dropping funnel, magnetic stirrer and a gas inlet adapter, from which glass tubing extends to the bottom of the flask, was condensed liquid ammonia until there was approximately 20 mL of anhydrous ammonia in the flask. To this dry ice/acetone cooled flask was added triethylchlorosilane (13.5 g, 89.4 mmol) dropwise over 30 min. After stirring for 1 h, excess ammonia was removed by placing the flask in a warm H_2O bath (< 40 °C). The remaining thick white suspension was extracted with dry ether (4 x 160 mL) and the solid was removed by filtration. The ether was then evaporated and the remaining liquid was fractionally distilled to give **23** (5.9 g, 60 %) as a colorless liquid: bp 134-135 °C (750 Torr) (lit.[264] bp 134 °C, 760 Torr); IR (neat film) 2950, 1237, 739 cm^{-1}; ^1H NMR (CD_2Cl_2, 400 MHz) δ 0.94 (t, 9 H, J = 8.0 Hz, $(C\underline{H}_3CH_2)_3Si$), 0.50 (q, 6 H, J = 8.0 Hz, $(CH_3C\underline{H}_2)_3Si$), 0.35-0.13 (br s, 2 H, $N\underline{H}_2$); ^{13}C NMR (CD_2Cl_2, 100 MHz) δ 7.1 ($(\underline{C}H_3CH_2)_3Si$), 6.2 ($(CH_3\underline{C}H_2)_3Si$); HRMS (EI) Calcd for $C_6H_{17}NSi$ 131.1130, found 131.1141.



Representitive experiment: N^{α}-(Benzyloxycarbonyl)-β-*N,N*-dimethylamino-L-alanine (24). To a stirred solution of *N,N*-dimethyl(trimethylsilyl)amine **19** (76 mg, 0.65 mmol) in dry CH_3CN (3 mL) was added *N*-Cbz-L-serine β-lactone **7** (111 mg, 0.50 mmol) in dry CH_3CN (2 mL) under argon. The reaction mixture was stirred for 2 h, at which point tlc indicated complete consumption of the β-lactone. The solution was then cooled on ice and cold 0.1 M HCl (10 mL) was added in one portion. The cloudy mixture was allowed to warm to room temperature and was stirred vigorously for another 30 min. Extraction with CH_2Cl_2 (3 x 10 mL) and evaporation of the aqueous phase *in vacuo* gave a white foam. This was purified by MPLC (MeOH-H_2O, 3:7) to give **24** (117 mg, 88 %) as a solid. A small sample was recrystallized from a methanol-ether mixture (1 : 3) to give **24** as a colorless crystalline solid: mp 150-152 °C (dec); IR (MeOH cast) 3600-2000 (br), 1711, 1620 cm^{-1}; ^1H NMR (D_2O, 360 MHz) δ 7.45 (s, 5 H, Ar$\underline{H}$), 5.15 (s, 2 H, $PhC\underline{H}_2O$), 4.40 (br dd, 1 H, J = 8.1, 6.2 Hz, $-C\underline{H}_{\alpha}$), 3.50 (dd, 1 H, J = 12.8, 6.0 Hz, $(CH_3)_2N-C\underline{H}H-$), 3.35 (dd, 1 H, J = 12.9, 10.0 Hz, $(CH_3)_2N-CH\underline{H}-$), 2.93 (s, 6 H, $(C\underline{H}_3)_2N$); ^{13}C NMR (CD_3OD, 100 MHz) δ 174.6 ($\underline{C}$=O acid), 158.5 ($\underline{C}$=O urethane), 138.0 (Ar$\underline{C}$), 129.5, 129.1 and 129.0 (Ar$\underline{C}$H), 68.0 (Ph$\underline{C}H_2O$), 60.7 ($\underline{C}H_2N$), 51.8 ($\underline{C}H_\alpha$), 43.9 ($N(\underline{C}H_3)_2$); MS (CI, NH_3) *m/z* (relative intensity) 267 (MH^+, 100%); HRMS (EI) Calcd for $C_{13}H_{18}N_2O_4$ 266.1267, found 266.1266 and for $C_{12}H_{18}N_2O_2$ $(M-CO_2)^+$ 222.1368, found 222.1369.

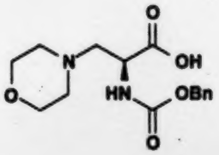


N^α-(Benzyloxycarbonyl)-β-*N,N*-diethylamino-L-alanine (25). The procedure outlined for synthesizing compound **24** was followed for the preparation of compound **25**. Thus, the reaction of *N,N*-diethyltrimethylsilylamine **20** (95 mg, 0.65 mmol) with *N*-Cbz-L-serine β-lactone **7** (111 mg, 0.50 mmol) gave the crude product. Purification by MPLC (MeOH-H_2O, 3:7) gave the desired compound **25** (115 mg, 78%) as a solid: mp 113-115 °C; IR (CH_2Cl_2 cast) 3600-2000 (br), 1713, 1623 cm^{-1}; ^1H NMR (D_2O, 360 MHz) δ 7.45 (s, 5 H, Ar$\underline{H}$), 4.35 (t, 1 H, *J* = 7.0 Hz, C$\underline{H}_\alpha$), 3.48 (dd, 1 H, *J* = 12.9, 6.4 Hz, Et_2N-C$\underline{H}$H) , 3.43 (dd, 1 H, *J* = 13.0, 8.6 Hz, Et_2N-CH$\underline{H}$-), 3.25 (br q, 4 H, *J* = 7.1 Hz, $(CH_3C\underline{H}_2)_2N$) 1.30 (t, 6 H, *J* = 7.0 Hz, $(C\underline{H}_3CH_2)_2N$); ^{13}C NMR (D_2O, 100 MHz) δ 174.3 ($\underline{C}$=O acid), 157.6 ($\underline{C}$=O urethane), 136.0 (Ar$\underline{C}$), 128.6, 128.3 and 127.7 (Ar$\underline{C}$H), 67.2 (Ph$\underline{C}H_2O$), 52.7 (Et_2N-$\underline{C}H_2$), 50.7 ($\underline{C}H_\alpha$), 47.9 ($(CH_3\underline{C}H_2)_2N$-), 7.9 ($(\underline{C}H_3CH_2)_2N$-); MS (CI, NH_3) *m/z* (relative intensity) 295 (MH^+, 100%). Anal. Calcd for $C_{15}H_{22}N_2O_4$: C, 61.21; H, 7.53; N, 9.52. Found: C, 60.94; H, 7.16; N, 9.29.



N^α-(Benzyloxycarbonyl)-β-(1-pyrrolidinyl)-L-alanine (26). This compound was prepared in the same way as for the preparation of compound **24**. Thus, the reaction of 1-(trimethylsilyl)pyrrolidine (93 mg, 0.65 mmol) with *N*-Cbz-L-serine β-lactone **7** (111 mg, 0.50 mmol) gave, after MPLC purification (MeOH-H_2O, 3:7), **26** (108 mg, 74%) as a white solid: mp 158-160 °C (dec); IR (CH_2Cl_2 cast) 3600-2100 (br), 1713, 1621, 1532, 1497 cm^{-1}; ^{1}H NMR (D_2O, 360 MHz) δ 7.45 (s, 5 H, Ar$\underline{H}$), 5.15 (s, 2 H, PhC$\underline{H}_2$O), 4.35 (br t, 1 H, *J* = 7.2 Hz, C$\underline{H}_\alpha$), 3.56 (dd, 1 H, *J* = 12.6, 4.3 Hz, (CH_2-CH_2)$_2$N-C$\underline{H}$H), 3.50-3.20 (m, 5 H, (CH_2-CH_2)$_2$N-CH$\underline{H}$ and (CH_2-C$\underline{H}_2$)$_2$N), 2.10-1.90 (m, 4 H, (C$\underline{H}_2$-CH_2)$_2$N); ^{13}C NMR (CD_3OD, 100 MHz) δ 174.5 ($\underline{C}$=O acid), 158.5 ($\underline{C}$=O urethane), 138.0 (Ar$\underline{C}$), 129.5 129.1 and 129.0 (Ar$\underline{C}$H), 67.9 (Ph$\underline{C}H_2$O), 58.3 ((CH_2-CH_2)$_2$N-$\underline{C}H_2$-), 55.6 ((CH_2-$\underline{C}H_2$)$_2$N), 53.5 ($\underline{C}H_\alpha$), 24.0 (($\underline{C}H_2$-CH_2)$_2$N); HRMS (EI) Calcd for $C_{15}H_{20}N_2O_4$ 292.1423, found 292.1424. Anal. Calcd for $C_{15}H_{20}N_2O_4$: C, 61.63; H, 6.90; N, 9.58. Found: C, 61.26; H, 6.98; N, 9.40.

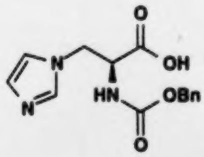
Reaction of the parent amine (pyrrolidine) with β-lactone **7** in CH_3CN gave the same compound but in only 45 % yield.



***N*$^{\alpha}$-(Benzyloxycarbonyl)-β-*N*-morpholino-L-alanine (27).** This alanine derivative was prepared by the same procedure used for the synthesis of compound **24**. Thus, the reaction of 4-(trimethylsilyl)morpholine (104 mg, 0.65 mmol) with *N*-Cbz-L-serine β-lactone **7** (111 mg, 0.50 mmol) yielded, after MPLC purification (MeOH-H_2O, 1:4), the desired compound **27** (120 mg, 78) as a white solid: mp 85-88 °C; IR (CH_2Cl_2 cast) 3600-3000 (br), 1709, 1619, 1529 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.40-7.25 (m, 5 H, Ar$\underline{H}$), 5.15 (s, 2 H, PhC$\underline{H}_2$O), 4.35 (t, 1 H, *J* = 7.3 Hz, C$\underline{H}_\alpha$), 3.82 (br s, 4 H, -C$\underline{H}_2$OC$\underline{H}_2$-), 3.21-3.10 (m, 6 H, NC$\underline{H}_2$ and -C$\underline{H}_2$NC$\underline{H}_2$-)); ^{13}C NMR (D_2O, 100 MHz) δ 174.1 ($\underline{C}$=O acid) 157.8 ($\underline{C}$=O urethane), 136.1 (Ar$\underline{C}$), 128.8, 128.5 and 127.9 (Ar$\underline{C}$H), 67.4 (Ph$\underline{C}H_2$O), 63.6 (-$\underline{C}H_2$O$\underline{C}H_2$-), 58.4 (-$\underline{C}H_2$N$\underline{C}H_2$-), 52.0 (N$\underline{C}H_2$), 50.4

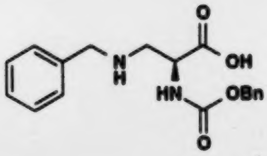
($\underline{C}H_{\alpha}$); MS (CI, NH_3) *m/z* (relative intensity) 309 (MH^+, 100%); HRMS (EI) Calcd for $C_{15}H_{20}N_2O_5$ 308.1372, found 308.1376.

Reaction of the parent amine (morpholine) with β-lactone **7** in CH_3CN gave the same compound but in only 36 % yield..



N

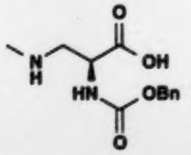
N^{α}-(Benzyloxycarbonyl)-β-imidazol-1-yl-L-alanine (28). This compound was prepared according to the procedure outlined for the preparation of compound **24**. Thus, 1-(trimethylsilyl)imidazole (91 mg, 0.65 mmol) was reacted with β-lactone **7** (111 mg, 0.50 mmol) to give, after MPLC purification (MeOH-H_2O, 1 : 4), **28** (86 mg, 60 %) as a white foam: IR (MeOH cast) 3570-2500 (br), 1710, 1615 cm $^{-1}$; ^1H NMR (CD_3OD, 200 MHz) δ 8.50 (s, 1 H, $\underline{H}$-2'), 7.35 (s, 1 H, $\underline{H}$-4' or $\underline{H}$-5'), 7.22-7.34 (m, 6 H, 5 Ar$\underline{H}$ and $\underline{H}$-4' or $\underline{H}$-5'), 5.08 (s, 2 H, PhC$\underline{H}_2$O), 4.65 (m, 1 H, C$\underline{H}_{\alpha}$), 4.45 (m, 2 H, imidazol-C$\underline{H}_2$); ^{13}C NMR (CD_3OD, 75 MHz) δ 173.6 ($\underline{C}$=O acid), 158.1 ($\underline{C}$=O urethane), 138.1 and 137.5 (Ar$\underline{C}$ and $\underline{C}$-2'), 129.5, 129.1 and 128.9 (Ar$\underline{C}$H), 123.3 and 122.8 ($\underline{C}$-3' and $\underline{C}$-4'), 67.7 (Ph$\underline{C}H_2$O), 57.3 ($\underline{C}H_{\alpha}$), 51.5 ($\underline{C}H_2$N); MS (CI, NH_3) *m/z* (relative intensity) 290 (MH^+, 31%); HRMS (EI) Calcd for $C_{14}H_{15}N_3O_4$ 289.1063, found 289.1061.



N^{α}-(Benzyloxycarbonyl)-β-benzylamino-L-alanine (29). The preparation of this compound was conducted according to the procedure outlined for the synthesis of compound **24**. Thus, the reaction of *N*-benzyl(trimethylsilyl)amine **21** (108 mg, 0.60 mol) with *N*-Cbz-L-serine β-lactone **7** (111 mg, 0.50 mmol) in CH_3CN for 12 h at 20 °C

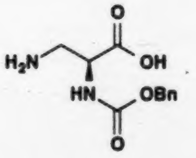
produced **29** as a white solid. This material was purified by recrystallization from MeOH / ether to give pure **29** as a white, fluffy solid (139 mg, 85 %): mp 185-186 °C (dec); IR (KBr) 3600-2200 (br), 1708, 1640, 1531, 696 cm^{-1}; ^{1}H NMR (DMSO-d_6, 360 MHz) δ 7.36 (m, 10 H, Ar$\underline{H}$), 7.10 (d, 1 H, *J* = 7.2, $\underline{H}NCO_2Bn$), 5.07 (s, 2 H, PhC$\underline{H}_2$O), 3.95 (m, 3 H, BnC$\underline{H}_2$N and C$\underline{H}_\alpha$), 3.45 (br s, 2 H, exchangeable N$\underline{H}_2$), 2.95 (dd, 1 H, *J* = 12.0, 6.1 Hz, BnNHC$\underline{H}$H-), 2.80 (dd, 1 H, *J* = 12.1, 8.9 Hz, BnNHCH$\underline{H}$-); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.9 ($\underline{C}$=O acid), 155.9 ($\underline{C}$=O urethane), 136.9 and 136.0 (Ar$\underline{C}$), 128.8, 128.5, 128.3, 127.8, 127.7 and 127.6 (Ar$\underline{C}$H), 65.4 (Ph$\underline{C}$H$_2$O), 51.0 ($\underline{C}H_\alpha$), 50.6 ($\underline{C}H_2N$), 47.9 ($\underline{C}H_2N$); MS (FAB, Cleland) *m/z* (relative intensity) 329.02 (MH$^+$, 51%); HRMS (EI) Calcd for $C_{18}H_{20}N_2O_4$ 328.1423, found 328.1422 and for $C_{11}H_{12}N_2O_3$
(M-PhCH$_2$OH)$^+$ 220.0848, found 220.0845. Anal. Calcd for $C_{18}H_{20}N_2O_4$: C, 65.84; H, 6.14; N, 8.53. Found: C, 65.68; H, 6.14; N, 8.52.

Reaction of the parent amine (benzyl amine) with β-lactone **7** in CH$_3$CN gave the same compound but in 60 % yield.

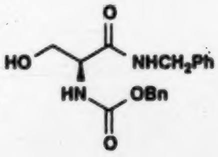


N^α-(Benzyloxycarbonyl)-β-*N*-methylamino-L-alanine (30). This compound was prepared by the same method outlined above for the preparation of **24**. Thus, the reaction of *N*-methyl(trimethylsilyl)amine **22** (52 mg, 0.50 mmol) with β-lactone **7** (66 mg, 0.30 mmol) for 1 h afforded, after MPLC purification (MeOH-H$_2$O , 3 :7), **30** (53 mg, 70 %) as a white solid: mp 160-162 °C (dec); IR (KBr) 3600-2000 (br), 1714, 1616, 1530 cm^{-1}; ^{1}H NMR (D$_2$O, 360 MHz) δ 7.45 (s, 5 H, Ar$\underline{H}$), 5.16 (s, 2 H, PhC$\underline{H}_2$O), 4.35 (br dd, 1 H, *J* = 8.8, 5.0 Hz, C$\underline{H}_\alpha$), 3.46 (dd, 1 H, *J* = 12.9, 5.0 Hz, CH$_3$NHC$\underline{H}$H-), 3.27 (dd, 1 H, *J* = 13.0, 8.6 Hz, CH$_3$NHCH$\underline{H}$-), 2.76 (s, 3 H, C$\underline{H}_3$-NH);

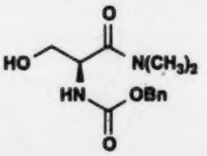
^{13}C NMR ($CD_3OD + D_2O$, 100 MHz) δ 174.1 (C=O acid), 158.7 (C=O urethane), 137.7 (ArC), 129.6, 129.2 and 129.0 (ArCH), 68.1 ($PhCH_2O$), 53.2 (CH_α), 52.1 (NCH_2-), 34.0 (CH_3NH); HRMS (EI) Calcd for $C_{12}H_{16}N_2O_4$ 252.1105, found 252.1105.

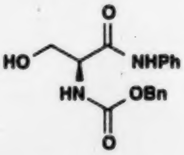


(2*S*)-*N*$^\alpha$-(Benzyloxycarbonyl)-2,3-diaminopropanoic acid (31). To a stirred solution of triethylsilylamine **23** (85 mg, 0.65 mmol) in dry CH_3CN (5 mL) was added β-lactone **7** (111 mg, 0.500 mmol) in dry CH_3CN (2 mL) and the mixture was stirred at 50 °C for 18 h. The reaction mixture was then cooled and was worked-up in the usual way. Purification by MPLC (MeOH-H_2O, 1 : 4) gave **31** (53 mg, 45 %) as a white solid: mp 229-230 °C (dec) (lit. mp 229-231 °C); IR (KBr) 3303, 3000-2100 (br), 1694, 1592, 1273 cm^{-1}; ^{1}H NMR (D_2O + DCl, 200 MHz) δ 7.35 (s, 5 H, ArH), 5.09 (s, 2 H, $PhCH_2O$), 4.54 (dd, 1 H, *J* = 8.9, 5.0 Hz, CH_α), 3.54 (m, 1 H, $CHHNH_3^+$), 3.29 (m, 1 H, $CHHNH_3^+$); HRMS (EI) Calcd for $C_{11}H_{14}N_2O_4$ 238.0954, found 238.0953.

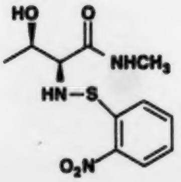


Preparation of authentic serineamides; representitive experiment: *N*-(benzyloxycarbonyl)-L-serine *N*-benzylamide (32). This L-serine *N*-benzylamide derivative was prepared by modifying the literature method developed for peptide coupling. To a solution of *N*-Cbz-L-serine (0.50 g, 2.1 mmol) in dry THF (10 mL) was added freshly distilled benzylamine (0.44 mL, 4.0 mmol) followed immediately by DCC (0.45 g, 2.2 mmol). The resulting white suspension was stirred at room temperature for 20 h then, was filtered. The solid collected was washed with THF (3 x 10 mL) and dried *in vacuo*. This material was recrystallized from EtOAc / hexane to afford **32** (570 mg, 83 %) as a white solid: mp 153-154 °C (dec); IR (KBr) 3380, 3100-2800 (br), 1705, 1640, 1576, 1046 cm^{-1}; ^{1}H NMR (DMSO-d_6, 360 MHz) δ 7.48-7.35 (m, 10 H, Ar$\underline{H}$), 6.58 (d, 1 H, J = 6.8 Hz, N$\underline{H}$), 5.03 (s, 2 H, PhC$\underline{H}_2$O), 3.93 (s, 2 H, PhC$\underline{H}_2$N), 3.70 (q, 1 H, J = 6.2 Hz, C$\underline{H}_\alpha$), 3.77 (dd, 1 H, J = 10.1, 5.4 Hz, HO-C$\underline{H}$H), 3.47 (dd, 1 H, J = 10.4, 6.4 Hz,HO-CH$\underline{H}$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 172.9 ($\underline{C}$=O amide), 155.6 ($\underline{C}$=O urethane), 137.2, 136.7 (Ar$\underline{C}$), 128.4, 128.4, 128.3, 127.8, 127.7 and 127.6 (Ar$\underline{C}$H), 65.2 and 62.3 ($\underline{C}$H$_2$-OH and Ph$\underline{C}$H$_2$O), 56.3 ($\underline{C}$H$_\alpha$), 42.9 (Ph$\underline{C}$H$_2$N); HRMS (EI) Calcd for $C_{11}H_{13}O_5N$ (M - PhCH$_2$)$^+$ 239.0794, found 239.0800.

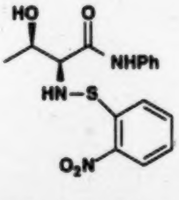




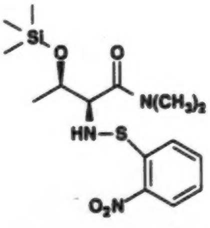
***N*-(Benzyloxycarbonyl)-L-serine *N,N*-dimethylamide (16), and *N*-(benzyloxycarbonyl)-L-serinephenylamide (18).** These authentic compounds were prepared according to the procedure outlined above for **32** in 88 % and 81 % yields, respectively. Spectroscopic data for these compounds was consistent with that given above for **16** and **18** prepared by the β-lactone route.

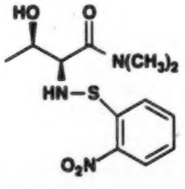


***N*-[(*o*-Nitrophenyl)sulfenyl]-L-threonine *N*-methylamide (33).** To a stirred suspension of methylamine hydrochloride (68 mg, 1.00 mmol) in dry CH$_2$Cl$_2$ (4 mL) was added trimethylaluminium (0.50 mL of a 2.0 M solution in hexane, 1.00 mmol) at 0 °C. After the addition was complete, the cloudy suspension was warmed to room temperature and stirred for an additional 50 min, during which time the solid material dissolved. To this solution at 0 °C was added β-lactone **11** (127 mg, 0.50 mmo) in dry CH_2Cl_2 (2 mL). The clear yellow solution was then allowed to warm to room temperature and stirring was continued whilst monitoring the consumption of the β-lactone by tlc. After 84 h, tlc still showed the presence of some β-lactone. The reaction was worked up in the same way as for the preparation of **33** to give a brown residue which was purified by flash chromatography (SiO_2, hexane-EtOAc, gradient, 9:1 to 1:3) to give **34** (60 mg, 57 % based on consumed starting material) as a yellow solid : mp 154-155 °C; IR (CH_2Cl_2 cast) 3600-3000 (br), 1652, 1507 cm^{-1}; ^1H NMR (CD_3COCD_3, 400 MHz) δ 8.30-8.26 (m, 1 H, ArH), 8.15-8.11 (m, 1 H, ArH), 7.79-7.75 (m, 1 H, ArH), 7.40-7.36 (m, 1 H, ArH), 7.31 (br s, 1 H, amide NH), 4.47 (d, 1 H, J = 4.6 Hz, OH), 4.45 (d, 1 H, J = 4.9 Hz, HN-SAr), 4.06 (m, 1 H, CH_β), 3.30 (t, 1 H, J = 11.4 Hz, -CH_α), 2.76 (d, 3 H, J = 5.1 Hz, CH_3-NH), 1.22 (d, 3 H, J = 5.9 Hz, CH_3CH-OH); ^{13}C NMR (CD_3COCD_3, 75 MHz) δ 172.4 (C=O), 146.4 and 143.6 (2 ArC), 134.8, 126., 125.9 and 125.8 (4 ArCH), 70.5 and 69.2 (CHOH and CHN), 26.0 (CH_3N), 19.5 (CH_3-CH-OH); HRMS (EI) Calcd for $C_{11}H_{15}N_3O_4S$ 285.0783, found 285.0779. Anal. Calcd for $C_{11}H_{15}N_3O_4S$: C, 46.32; H, 5.30; N, 14.73. Found: C, 46.63; H, 5.26; N, 14.33.



N-[(*o*-Nitrophenyl)sulfenyl]-L-threonine N-phenylamide (34). To a stirred solution of aniline (93 mg, 1.0 mmol) in dry CH_2Cl_2 (4 mL) was added dimethylaluminium chloride (1.00 mL of a 1.0 M solution in hexane, 1.00 mmol) at 0 °C. After the addition was complete, the pale yellow solution was allowed to warm to room temperature and was stirred for a further 10 min. The reaction mixture was then cooled to 0 °C and β-lactone **11** (127 mg, 0.50 mmol) in dry CH_2Cl_2 (2 mL) was added. The resulting pale green solution was warmed to room temperature and stirring was continued while monitoring the consumption of the β-lactone by tlc. After 17 h, tlc indicated that some of the β-lactone was still present, however the reaction was worked-up by first cooling to 0 °C and then slowly adding 0.1 M HCl (15 mL, pre-cooled to 4 °C). After vigorous stirring for 30 min at room temperature, the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (4 x 15 mL). The combined organic extracts were washed with H_2O (40 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give an orange residue. The residual oil was purified by flash chromatography (SiO_2, petroleum ether-EtOAc, gradient, 4:1 to 1:1) to give **33** (69 mg, 40 %) as a yellow solid: mp 162-164 °C; IR (KBr) 3600-3000 (br), 1655, 1512 cm^{-1}; ^1H NMR (CD_3COCD_3, 400 MHz) δ 9.35 (s, 1 H, amide N$\underline{H}$), 8.28-8.24 (m, 2 H, Ar$\underline{H}$), 7.75 (m, 1 H, Ar$\underline{H}$), 7.62 (m, 1 H, Ar$\underline{H}$), 7.38-7.20 (m, 3 H, Ar$\underline{H}$), 7.07 (m, 1 H, Ar$\underline{H}$), 4.68 (d, 1 H, J = 4.6 Hz, $\underline{H}$O-CH), 4.58 (d, 1 H, J = 6.5 Hz, CH-N$\underline{H}$-S), 4.23 (m, 1 H, C$\underline{H}_\beta$), 3.55 (dd, 1 H, J = 6.5 , 5.2 Hz, -C$\underline{H}_\alpha$), 1.32 (d, 3 H, J = 6.2 Hz, C$\underline{H}_3$); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ 170.9 ($\underline{C}$=O), 146.3, 143.6 and 139.5 (Ar$\underline{C}$), 134.9, 129.6, 126.4, 125.8, 124.6 and 120.5 (Ar$\underline{C}$H); 71.6 and 69.4 ($\underline{C}$HOH and $\underline{C}$HN), 19.8 ($\underline{C}H_3$); MS (FAB, Cleland) *m/z* (relative intensity) 348.12 (MH^+, 33%); HRMS (EI) Calcd for $C_{16}H_{17}N_3O_4S$ 347.0940, found 347.0936.





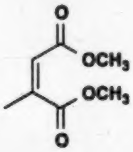
***N*-[(*o*-Nitro-phenyl)sulfenyl]-*O*-(trimethylsilyl)-L-threonine *N,N*-dimethyl-amide (35) and *N*-[(*o*-Nitrophenyl)sulfenyl]-L-threonine *N,N*-dimethylamide (36).** To a stirred solution of *N,N*-dimethyl(trimethylsilyl)amine **19** (141 mg, 1.20 mmol) in dry CH_3CN (4 mL) was added β-lactone **11** (254 mg, 1.00 mmol) in dry CH_3CN (2 mL) at room temperature under argon. The reaction mixture was then heated to 50 °C and stirring was continued for 48 h. The resulting orange solution was cooled to 0 °C and 0.1 M HCl (15 mL) was added in one portion. After stirring for 15 min, the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 150 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give an orange oil. Purification by flash chromatography (SiO_2, hexane-EtOAc, gradient, 4:1 to 1:4) afforded **35** (240 mg, 65 %) and **36** (38 mg, 13 %), both as yellow oils.

For **35**: IR (CH_2Cl_2 cast) 1642, 1592, 1510, 1339 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 200 MHz) δ 8.31 (m, 1 H, ArH), 8.26 (m, 1 H, ArH), 7.80-7.68 (m, 1 H, ArH), 7.40-7.30 (m, 1 H, ArH), 4.24-4.19 (m, 1 H, CH_3CH-OTMS), 4.16 (d, 1 H, *J* = 10.1 Hz, CHNH-S), 3.62 (dd, 1 H, *J* = 9.0, 4.5 Hz, -CH_{α}), 3.05 and 2.93 (2s, 6 H, $N(CH_3)_2$), 1.28 (d, 3 H, *J* = 6.0 Hz, CH_3CH), 0.14 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (CD_3COCD_3 + CD_3OD, 75 MHz) δ 172.9 (C=O amide), 146.7 and 143.4 (ArC), 134.7, 125.9, 125.7 and 125.6, (ArCH), 67.6 and 67.0 (CHOH and CHN), 37.6 and 35.7 ($N(CH_3)_2$), 19.6 (CH_3-CHOH), 0.1 ($Si(CH_3)_3$); MS (CI, NH_3) *m/z* (relative intensity) 372 (MH^+, 100%), 219 (38). Anal. Calcd for $C_{15}H_{25}N_3O_4SSi$: C, 48.49; H, 6.78; N, 11.31; S, 8.63. Found: C, 48.53; H, 7.05; N, 11.21; S, 8.81.

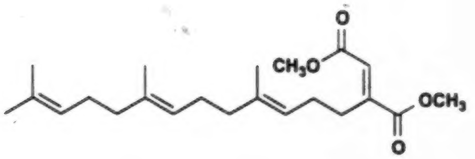
For **36**: IR ($CHCl_3$ cast) 3600-3000 (br), 1628, 1507, 1337 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 200 MHz) δ 8.28-8.23 (m, 2 H, ArH), 7.84-7.79 (m, 1 H, ArH), 7.48-7.42 (m, 1 H, ArH), 4.35 (d, 1 H, *J* = 8.6 Hz, CHNH-S), 4.35-4.15 (br s, 1 H, OH), 4.17-4.02 (m, 1 H, CH_{β}), 3.90 (dd, 1 H, *J* = 9.0, 4.9 Hz, CH_{α}), 3.05 and 2.97 (2s, 6 H, $N(CH_3)_2$), 1.30 (d, 3 H, *J* = 6.3 Hz, CH_3CH-OH); ^{13}C NMR (CD_3OD, 75 MHz) δ 173.9 (C=O amide), 146.8 and 143.4 (ArC), 134.7, 126.3, 125.9 and 125.7 (ArCH), 70.2 and 68.0 (CHOH and CHN), 37.8 and 36.2 ($N(CH_3)_2$), 19.8 (CH_3CH-OH); MS (CI, NH_3) *m/z* (relative intensity) 300 (MH^+, 100%), 147 (80). Anal. Calcd for $C_{12}H_{17}N_3O_4S$: C, 48.15; H, 5.72; N, 14.04; S, 10.71. Found: C, 48.21; H, 5.77; N, 13.69; S, 10.62.

Compound **35** was deprotected using *tetra-n*-butylammonium fluoride (TBAF) to give **36** according to the following procedure. To a stirred solution of **35** (46 mg, 0.12

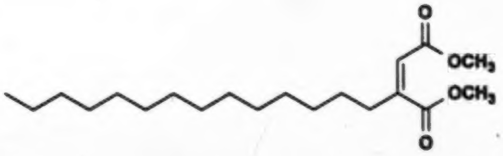
mmol) in dry THF (2.5 mL) was added TBAF (0.36 mL, 0.36 mmol, 1 M solution in THF) at 20 °C. After stirring for 45 min, the mixture was concentrated *in vacuo* to afford a brown residue. Purification by flash chromatography (SiO_2, 100 % EtOAc (150 mL) then 10 % $CHCl_3$ in EtOAc (200 mL)) gave **36** (30 mg, 84 %) as a yellow oil. The spectroscopic data for this compound was identical with that given above.



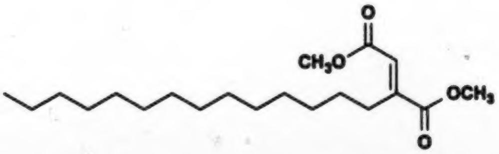
Dimethyl (*Z*)-2-methylbutenedioate (37). A mixture of citraconic anhydride (50.0 g, 0.451 mmol), methanol (200 mL) and *p*-toluenesulfonic acid (800 mg) was heated at reflux for 20 h. After the mixture was cooled, toluene (100 mL) was added and the azeotropic mixture consisting of methanol, toluene and water was distilled. Fresh dry methanol (200 mL) was added to the remaining oil and the mixture was heated at reflux for an additional 18 h. After adding toluene (100 mL) and distilling the solvent as before, the residual liquid was diluted with ether (100 mL) and washed with 5 % $NaHCO_3$ (2 x 50 mL), H_2O (50 mL) and brine (50 mL). Drying (Na_2SO_4) and concentration *in vacuo* gave a yellow liquid which was distilled at reduced pressure to give the title compound **37** (60.5 g, 85 %) as a colorless liquid: bp 91-92 °C (0.65 torr); IR (neat film) 2955, 1732, 1655, 1448, 1436, 1362, 1274 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 5.69 (q, 1 H, $J = 1.5$ Hz, $C{=}C\underline{H}$), 3.62 and 3.53 (2s, 6 H, 2 x $OC\underline{H}_3$), 1.87 (d, 3 H, $J = 1.7$ Hz, $C\underline{H}_3C{=}C$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 168.8 and 164.8 (2 x $\underline{C}{=}O$), 145.3 ($\underline{C}{=}CH$), 120.2 ($C{=}\underline{C}H$), 51.7 and 51.2 (2 x $O\underline{C}H_3$), 19.8 ($\underline{C}H_3C{=}CH$).; HRMS (EI) Calcd for $C_7H_{10}O_4$ 158.0579, found 158.0579. Anal. Calcd for $C_7H_{10}O_4$: C, 53.16; H, 6.37. Found: 53.32; H, 6.24.



Dimethyl (*E*)-2-homofarnesylbutenedioate (38). To lithium hexamethyldisilazane (1.60 mL of a 1.0 M solution in THF, 1.60 mmol) was added a solution of **37** (237 mg, 1.50 mmol) in THF (2 mL) at -78 °C and the mixture was stirred for 5 min. To the resulting deep yellow solution was then added trimethylsilyl chloride (174 mg, 1.60 mmol) and the mixture was allowed to warm to room temperature over 1 h and stirred for 30 min. Farnesyl bromide (526 mg, 1.84 mmol) was added followed by dry zinc bromide (15 mg) and the mixture was stirred for 1 h. Aqueous work-up and purification by flash column chromatography (SiO_2, petrolum ether-ether, 10:0 to 5:5) gave **38** as the only identifiable product (7.0 mg, 1.3 %): IR ($CDCl_3$ cast) 2945, 2915, 1727 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 6.75 (s, 1 H, $C{=}C\underline{H}$), 5.19-5.02 (m, 3 H, 3 x $C{=}C\underline{H}$), 3.78 and 3.74 (2s, 6 H, 2 x $OC\underline{H}_3$), 2.83 (t, 2 H, *J* = 7.4 Hz, $-CH_2C\underline{H}_2C{=}CCO$), 2.18 (q, 2 H, *J* = 7.6 Hz, $-C\underline{H}_2CH_2C{=}CCO$), 2.10-1.91 (m, 8 H, 2 $(C\underline{H}_2)_2$), 1.68 (s, 3 H, $C\underline{H}_3$-chain), 1.60 (s, 9 H, 3 $C\underline{H}_3$-chain); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.4 and 166.0 (2 x $\underline{C}{=}O$), 147.7, 136.5, 135.0 and 131.2, (4 x $\underline{C}{=}C$), 126.4, 124.4, 124.2 and 122.8 (4 x $C{=}\underline{C}H$), 52.4 and 51.6 (2 x $O\underline{C}H_3$), 39.7 ($CH_2\underline{C}H_2C{=}CCO$), 28.0, 27.5, 26.8 and 26.7 (2 x $(\underline{C}H_2)_2$ + $\underline{C}H_2CH_2C{=}CCO$), 25.7, 17.7, 16.0 and 15.9 (4 x $\underline{C}H_3$-chain); HRMS (EI) Calcd for $C_{22}H_{34}O_4$ 362.2457, found 362.2456.

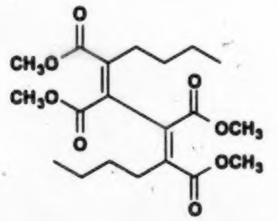


Conjugate addition to dimethyl acetylenedicarboxylate (DMAD). Dimethyl (*Z*)-2-tetradecylbutenedioate (40a). Tetradecylmagnesium chloride (1.20 mL of a 1.0 M solution in THF, 1.2 mmol) was added dropwise to a suspension of cuprous bromide-dimethyl sulfide complex, CuBr•Me_2S, (0.25 g, 1.20 mmol) in THF (6 mL) at -40 °C. The resulting yellow suspension was stirred at - 40 °C for 2 h, then cooled to -78 °C and freshly distilled dimethyl acetylenedicarboxylate (DMAD) (0.14 g, 1.00 mmol) in THF (2 mL) was added dropwise to give a dark red brown mixture. After 1 h, the reaction mixture was quenched with saturated aqueous NH_4Cl solution (2 mL, adjusted to pH 8 with 10% ammonia) and allowed to warm to room temperature. After 30 min, the mixture was partitioned between ether and water. The aqueous layer was extracted with ether (3 x 5 mL) and the combined organic extracts were washed with saturated aqueous NH_4Cl solution (20 mL) and brine (20 mL). Drying over Na_2SO_4 and concentration *in vacuo* gave 332 mg of crude product. Purification by flash column chromatography (SiO_2, petroleum ether-ether, 9:1) gave **40a** (289 mg, 85 %) as a white solid: mp 51-52°C; IR ($CDCl_3$ cast) 2916, 2849, 1729, 1717 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 5.81 (t, 1 H, J = 1.4 Hz, C=C$\underline{H}$), 3.83 and 3.72 (2s, 6 H, 2 x OC$\underline{H}_3$), 2.35 (dt, 2 H, J = 8.3, 1.4 Hz, $CH_2C\underline{H}_2C$=C), 1.50 (qn, 2 H, $C\underline{H}_2CH_2C$=C), 1.3 (br m, 22 H, $(C\underline{H}_2)_{11}$), 0.88 (t, 3 H, J = 6.6 Hz, $C\underline{H}_3$-chain); ^{13}C NMR ($CDCl_3$,100 MHz) δ 169.4 and 165.4 (2 x $\underline{C}$=O), 151.1 ($\underline{C}$=CH), 119.0 (C=$\underline{C}$H), 52.3 and 51.8 (2 x $O\underline{C}H_3$), 34.4, 31.9, 29.6, 29.5, 29.4, 29.3,29.2, 28.9, 26.9 and 22.7 ($-(\underline{C}H_2)_{13}-$), 14.1 ($\underline{C}H_3$-chain); HRMS (EI) Calcd. for $C_{20}H_{36}O_4$ 340.2614, found 340.2614. Anal. Calcd for $C_{20}H_{36}O_4$: C, 70.55; H, 10.66. Found: C, 70.63; H, 10.71.

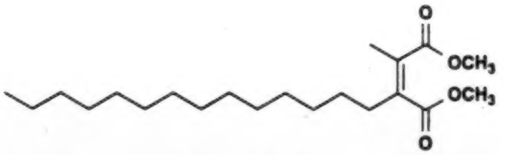


Dimethyl (Z)-2-tetradecylbutenedioate (40b). When the reaction was performed at higher temperature or in ether as a solvent (see discussion), the (*E*)-isomer **40b** was also obtained: IR ($CHCl_3$ cast) 2924, 2853, 1727, 1610 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.72 (s, 1 H, C=C$\underline{H}$), 3.80 and 3.75 (2s, 6 H, 2 x OC$\underline{H}_3$), 2.78 (t, 2 H, J = 7.5 Hz,

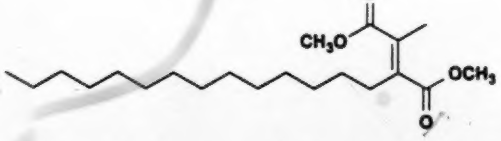
$CH_2C\underline{H}_2C{=}C$), 1.45 (qn, 2 H, $C\underline{H}_2CH_2C{=}C$), 1.25 (br s, 22 H, $(C\underline{H}_2)_{11}$), 0.88 (t, 3 H, J = 6.5 Hz, $C\underline{H}_3$-chain); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 168.2 and 166.1, (2 x $\underline{C}{=}O$), 148.6 ($\underline{C}{=}CH$), 126.0 ($C{=}\underline{C}H$), 52.5 and 51.7 (2 x $O\underline{C}H_3$), 31.9, 29.7, 29.6, 29.4, 29.3, 28.0 and 22.7 (-($\underline{C}H_2)_{13}$-), 14.1 ($\underline{C}H_3$-chain); HRMS (EI) Calcd. for $C_{20}H_{36}O_4$ 340.2614, found 340.2617. Anal. Calcd for $C_{20}H_{36}O_4$: C, 70.55; H, 10.66. Found: C, 70.80; H, 10.68.



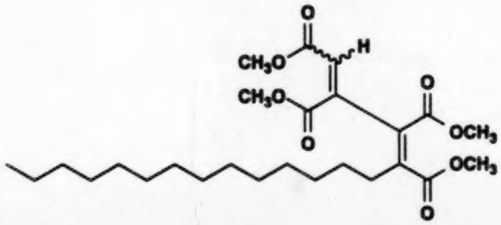
(Z,Z) Dimethyl 2,5-dibutyl-3,4-dicarbomethoxy-2,4-hexadiene-1,6-dioate (41). To a suspension of $CuBr.Me_2S$ (205 mg, 1.00 mmol) in THF (5 mL) was added n-BuLi (1.25 mL of a 1.6 M in hexane, 2.00 mmol) at - 78 °C over 5 min. After stirring the mixture for 45 min at - 78 °C, DMAD (115 mg, 0.800 mmol) in THF (2 mL) was added. The mixture was stirred for 45 min and quenched by the addition of saturated aqueous NH_4Cl solution (2 mL). Isolation as above gave a yellow residue which was subjected to flash column chromatography (SiO_2, petrolum ether-ether, 8:2) to afford **41** (121 mg, 38 %) as a colorless oil: IR (CH_2Cl_2 cast) 2934, 1728, 1620 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 3.80 and 3.70 (2s, 12 H, 4 x $OC\underline{H}_3$), 2.3-2.2 (m, 4 H, 2 x $C\underline{H}_2C{=}C$), 1.40-1.23 (m, 8 H, 2 x -$(C\underline{H}_2)_2$-), 0.85 (t, 6 H, J = 7.2 Hz, 2 x $C\underline{H}_3CH_2$-); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.2 and 165.2 (4 x $\underline{C}{=}O$), 148.8 and 125.8 (2 x $\underline{C}{=}\underline{C}$), 31.9, 28.8 and 22.5 (2 x -$(\underline{C}H_2)_3$-), 13.7 (2 x $\underline{C}H_3$); HRMS (EI) Calcd for $C_{20}H_{30}O_8$ 398.1941, found 398.1935.



General procedure for conjugate addition-enolate capture. Chaetomellic acid A dimethyl ester (42a). The procedure for **40a** was followed with the following modifications: after addition of DMAD at - 78 °C, the reaction mixture was stirred for 40 min, then a HMPA-THF solution (1:1, 2 mL) was added, which resulted in the heterogeneous mixture becoming nearly homogeneous. After 45 min the electrophile, MeI (0.360 g, 2.50 mmol) in THF (2 mL) was added and stirring was continued for 5 min at -78 °C. After warming the mixture to room temperature overnight, it was re-cooled to -20 °C, quenched with saturated aqueous NH_4Cl (2 mL, adjusted to pH 8 with 10 % ammonia) and allowed to warm to room temperature. The mixture was stirred at room temperature for 30 min, then partitioned between ether and water. The aqueous layer was extracted with ether (3 x 10 mL) and the combined organic extracts were successively washed with aqueous NH_4Cl (20 mL), water (2 x 20 mL) and brine (20 mL). Drying (Na_2SO_4) and concentration *in vacuo* gave 365 mg of a yellow oil. Purification by flash column chromatography (SiO_2; petroleum ether-ether, 8:2) gave **42a**[170] (277 mg, 78 %) as a colorless oil: IR ($CHCl_3$ cast) 2924, 2853, 1725, 1644, 1434 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.74 and 3.73 (2s, 6 H, 2 x $OC\underline{H}_3$), 2.31 (t, 3 H, J = 7.5 Hz, $CH_2C\underline{H}_2C{=}C$), 1.93 (s, 3 H, $C\underline{H}_3C{=}C$), 1.42 (qn, 2 H, $C\underline{H}_2CH_2C{=}C$), 1.24 (br m, 22 H, $(C\underline{H}_2)_{11}$), 0.86 (t, 3 H, J = 6.5 Hz, $C\underline{H}_3$-chain); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.6 and 169.1 (2 x $\underline{C}{=}O$), 139.7, 131.5 ($\underline{C}{=}\underline{C}$), 52.1 and 52.0 (2 x $O\underline{C}H_3$), 31.9, 30.1, 29.6, 29.5, 29.4, 29.3, 27.7 and 22.6 ($-(\underline{C}H_2)_{13}-$), 14.9, 14.0 (2 x $\underline{C}H_3$); HRMS (EI) Calcd for $C_{21}H_{38}O_4$ 354.2770, found 354.2763. Anal. Calcd for $C_{21}H_{38}O_4$: C, 71.15; H, 10.80. Found: C, 71.13; H, 10.77.



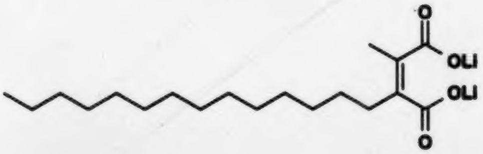
Dimethyl (*E*)-2-tetradecyl-3-methylbutenedioate (42b). Depending on the conditions (see discussion) the (*E*)-isomer **42b**[170] could also be isolated: IR ($CDCl_3$ cast) 2925, 2854, 1726, 1434 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.78 and 3.77 (2s, 6 H, 2 x $OC\underline{H}_3$), 2.43 (dt, 2 H, *J* = 7.6, 0.8 Hz, $CH_2C\underline{H}_2C{=}C$), 1.99 (s, 3 H, $C\underline{H}_3C{=}C$), 1.40 (qn, 2 H, $C\underline{H}_2CH_2C{=}C$), 1.25 (br m, 22 H, $(C\underline{H}_2)_{11}$), 0.87 (t, 3 H, *J* = 6.6 Hz, $C\underline{H}_3$-chain); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.5 and 169.3 (2 x $\underline{C}{=}O$), 139.1 and 131.9 ($\underline{C}{=}\underline{C}$), 51.8 and 51.7 (2 x $O\underline{C}H_3$), 31.9, 31.4, 29.7, 29.5, 29.4, 29.3, 29.2 and 22.7 (- $(\underline{C}H_2)_{13}$-), 17.6 ($\underline{C}H_3C{=}C$) 14.1 ($\underline{C}H_3$-chain); HRMS (EI) Calcd for $C_{21}H_{38}O_4$ 354.2770, found 354.2768. Anal. Calcd for $C_{21}H_{38}O_4$: C, 71.15; H, 10.80. Found: C, 71.28; H, 10.76.



Dimethyl 2-tetradecyl-3,4-dicarbomethoxy-2,4-hexadiene-1,6-dioate (43). The procedure for **40** was followed using ether as the reaction solvent and methyl triflate as the alkylating agent. Thus CuBr.Me_2S (411 mg, 2.00 mmol), tetradecylmagnesium chloride (2.00 mL of 1.0 M solution in THF, 2.00 mmol) and DMAD (284 mg, 2.00 mmol) were allowed to react as before. This was followed by the addition of HMPA (2 mL) and methyl triflate (679 mg, 6.00 mmol) in ether (5 mL). The reaction was stirred at -40 °C for 2 h and then warmed to room temperature overnight. The usual work up and purification gave **40b** (110 mg, 16 %) and **43** (339 mg, 35 %) as a colorless oil. For **43**: IR ($CHCl_3$ cast) 2952, 2925, 2854, 1728, 1627, 1434 cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 6.1 (s, 1 H, $C{=}C\underline{H}$), 3.82, 3.80, 3.78 and 3.73 (4s, 12 H, 4 x $OC\underline{H}_3$), 2.42 (t, 2 H, *J* = 7.6

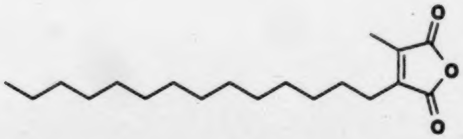
Hz, $-CH_2C\underline{H}_2C{=}C$), 1.41 (qn, 2 H, $-C\underline{H}_2CH_2C{=}C$)), 1.24 (br m, 22 H, $-(C\underline{H}_2)_{11}-$), 0.87 (t, 3 H, J = 6.5 Hz, $C\underline{H}_3$-chain); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.7, 165.1, 164.8 and 164.7 (4 x $\underline{C}{=}O$), 148.4, 137.8 and 127.9 ($\underline{C}{=}\underline{C}$ + $\underline{C}{=}CH$), 128.8 ($C{=}\underline{C}H$), 52.6, 52.5, 52.3 and 52.1 (4 x $O\underline{C}H_3$), 31.8, 29.5, 29.3, 29.2, 29.1, 27.5 and 22.5 ($-(\underline{C}H_2)_{13}$), 14.0 ($\underline{C}H_3$-chain); HRMS (EI) Calcd for $C_{26}H_{42}O_8$ 482.2880, found 482.2878.

General procedure for the basic hydrolysis of Chaetomellic acid A dimethyl ester 42a and derivatives to lithium salts, and for the formation of anhydrides. To the di-ester (50 mg) in THF-H_2O (2 mL, 1:1) was added 1.0 N LiOH (2 eq) and the mixture was stirred at room temperature and monitored for the consumption of starting material by tlc. The solvent was removed *in vacuo* and the remaining solid was dissolved in H_2O (3 mL). Non-polar impurities including unreacted starting material were removed by simple extraction of the aqueous layer with ether (3 mL). Freeze-drying of the aqueous layer gave the respective lithium salt. Alternatively, acidification of the aqueous solution with 1.0 N HCl at 0 °C and extraction with ether gave the corresponding anhydride which was purified if neccessary by flash column chromatography on silica.

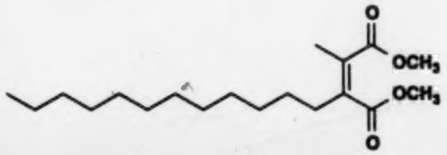


Chaetomellic acid A, dilithium salt (44). The hydrolysis of ester **42a** (50 mg, 0.14 mmol) gave salt **44** (44 mg, 99%) as a white solid: IR (KBr) 3440, 2921, 2851, 1555, 1438 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 2.24 (t, 2 H, J = 7.8 Hz, $-CH_2C\underline{H}_2C{=}C$), 1.83 (s, 3 H, $C\underline{H}_3C{=}C$), 1.47 (m, 2 H, $-C\underline{H}_2CH_2C{=}C$), 1.28 (br m, 22 H, $-(C\underline{H}_2)_{11}-$), 0.89 (t, 3 H, J = 6.8 Hz, $C\underline{H}_3$-chain); ^{13}C NMR (CD_3OD, 75 MHz) δ 180.2 and 179.9 (2 x $\underline{C}{=}O$), 139.6 and 132.8 ($\underline{C}{=}\underline{C}$), 33.1, 31.6, 31.1, 30.5, 30.4, 30.3, 29.5 and

23.7 (-($\underline{C}H_2$)$_{13}$), 16.3 and 16.2 (2 x $\underline{C}H_3$); MS (FAB, Cleland) *m/z* (relative intensity) 339 (MH^+, 9%). Anal. Calcd for $C_{19}H_{32}Li_2O_4.H_2O$: C, 64.04; H, 9.62. Found: C, 63.65; H, 9.36.

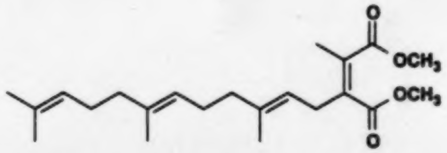


Chaetomellic acid A, anhydride (45). Direct acid work up of the solution of salt **44** and ether extraction gave anhydride **45** (43 mg, 99%) as a colorless oil: IR ($CHCl_3$ cast) 2924, 2853, 1767 cm^{-1}; ^{1}H NMR (CD_3OD, 360 MHz) δ 2.46 (t, 2 H, *J* = 7.5 Hz, -$CH_2\underline{H}_2C$=C), 2.03 (s, 3 H, $C\underline{H}_3C$=C), 1.57 (qn, 2 H, -$C\underline{H}_2CH_2C$=C), 1.34-1.28 (m, 22 H, -($C\underline{H}_2$)$_{11}$-), 0.89 (t, 3 H, *J* = 6.6 Hz, $C\underline{H}_3$-chain); ^{13}C NMR (CD_3OD, 75 MHz) δ 167.8 and 167.6 (2 x $\underline{C}$=O), 145.4 and 141.9 ($\underline{C}$=$\underline{C}$), 33.1, 30.8, 30.7, 30.6, 30.5, 30.4, 30.3, 28.5, 25.1 and 23.7 (-($\underline{C}H_2$)$_{13}$-), 14.4 ($\underline{C}H_3C$=C), 9.3 ($\underline{C}H_3$-chain); HRMS (EI) Calcd for $C_{19}H_{32}O_3$ 308.2351, found 308.2355. Anal. Calcd for $C_{19}H_{32}O_3$: C, 73.98; H, 10.46. Found: C, 73.67; H, 10.37.



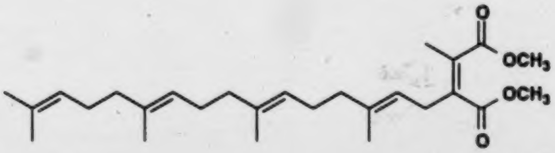
Dimethyl (*Z*)-2-Dodecyl-3-methylbutenedioate (46). The general procedure for **40** was followed using dodecylmagnesium bromide (1.20 mL of a 1.00 M in ether, 1.20 mmol), $CuBr.Me_2S$ (250 mg, 1.20 mmol), DMAD (140 mg, 1.00 mmol) and CH_3I (360 mg, 2.50 mmol) to give the crude product. Purification in the usual way (SiO_2, petrolum ether-ether, 8:2) gave **46** (250 mg, 77 %) as an oil: IR ($CHCl_3$ cast) 2925, 2854, 1725, 1643, 1459, 1434 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.73 and 3.71 (2s, 6 H, 2 x $OC\underline{H}_3$), 2.30 (t, 3 H, *J* = 7.3 Hz, $CH_2C\underline{H}_2C$=C), 1.91 (s, 3 H, $C\underline{H}_3C$=C), 1.38 (qn, 2 H, $C\underline{H}_2CH_2C$=C), 1.23 (br s, 18 H, ($C\underline{H}_2$)$_9$), 0.85 (t, 3 H, *J* = 6.2 Hz, $C\underline{H}_3$-chain); ^{13}C NMR

($CDCl_3$, 75 MHz) δ 169.6 and 169.0, (2 x C=O), 139.7 and 131.5 (C=C), 52.1 and 52.0 (2 x OCH_3), 31.9, 30.1, 29.6, 29.5, 29.4, 29.3 and 27.7 ($-(CH_2)_{11}-$), 14.9 and 14.0 (2 CH_3); HRMS (EI) Calcd for $C_{19}H_{34}O_4$ 326.2457 found 326.2457. Anal. Calcd for $C_{19}H_{34}O_4$: C, 69.90; H, 10.50. Found: C, 70.27; H, 10.83.

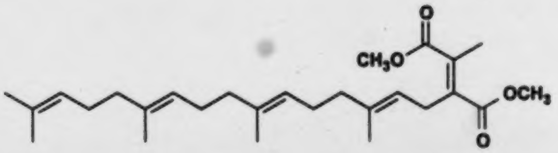


Dimethyl (*Z*)-2-farnesyl-3-methylbutenedioate (47). The general procedure for the preparation of **40** was employed: Thus $CuBr.Me_2S$ (0.41 g, 2.00 mmol), methyl magnesium bromide (0.67 mL of a 3.00 M solution in ether, 2.00 mmol), DMAD (0.27 g, 1.90 mmol) and farnesyl bromide (1.14 g, 4.00 mmol) were reacted as before. Purification of the crude product by flash column chromatography (SiO_2, petrolum ether-ether, 9:1) gave **47** (560 mg, 81 %) as an oil: IR (neat film) 2949, 2920, 1725, 1643, 1434 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.1 (m, 3 H, 3 x C=CH), 3.71 and 3.70 (2s, 6 H, 2 x OCH_3), 3.03 (d, 2 H, $J = 7.1$ Hz, $C=CHCH_2C=C$), 2.0 (m, 8 H, 2 x $(CH_2)_2$), 1.93 (s, 3 H, $CH_3C=CCO$) 1.71 and 1.65 (2s, 6 H, 2 x CH_3-chain) 1.56 (s, 6 H, 2 x CH_3-chain); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.2 and 169.0 (2 x C=O), 138.2, 138.1, 135.1, 132.0 and131.2 (5 x C=C), 124.1, 123.8 and 118.2 (3 x C=CH), 52.1 and 52.0 (2 x OCH_3), 39.6 ($C=CHCH_2C=C$), 28.9, 26.5, 26.3 and 25.7 (2 x $(CH_2)_2$), 17.6, 16.1, 16.0 and 15.1 (5 x CH_3); HRMS (EI) Calcd for $C_{22}H_{34}O_4$ 362.2457, found 362.2449. Anal. Calcd for $C_{22}H_{34}O_4$: C, 72.98; H, 9.45. Found: C, 72.92; H, 9.31.

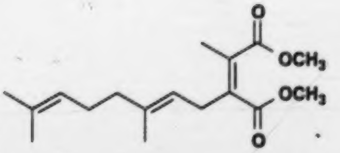
Dimethyl (*Z*)-2-farnesyl-3-methylbutenedioate (47) *via* palladium-mediated cross-coupling. To a degassed solution of farnesyl bromide (57.0 mg, 0.200 mmol) in dry DMF (1 mL) were added sequentially **53** (65.2 mg, 0.20 mmol) and $Pd(Ph_3)_4$ (23.0 mg, 0.020 mmol). Copper (I) iodide (29.0 mg, 0.15 mmol) was then added in one portion and the resulting yellow mixture was stirred under argon at room temperature for 12 h. The mixture was diluted with ether (15 mL) and filtered through Celite. The filtrate was stirred for 30 min with a large excess of a saturated aqueous NH_4Cl solution and the resultant organic phase was separated and concentrated *in vacuo*. The residue was diluted with ether (15 mL) and stirred for 1 h with 20 mL of a 50% aqueous KF solution. Filtration through Celite and extraction with ether (5 x 25 mL) gave the organic extracts which were washed with brine (50 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give an orange oil. Purification by flash chromatography (SiO_2, petrolum ether-ether, 8.5:1.5) gave **47** (62 mg, 85%) as a colorless oil whose spectral data were identical to those given above.

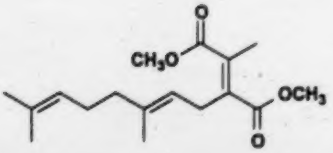


Dimethyl (Z)-2-(geranylgeranyl)-3-methylbutenedioate (48a). The general procedure for **40** was employed. Thus $CuBr.Me_2S$ (0.30 g, 1.46 mmol), methyl magnesium bromide (0.49 mL of a 3.00 M solution in ether, 1.46 mmol) and DMAD (0.20 g, 1.40 mmol) were allowed to react as before. Concurrently, geranylgeranyl bromide was being prepared according to a modified literature procedure. To a solution of geranylgeraniol (1.00 g, 3.44 mmol) in THF (5 mL) was added a solution of PBr_3 (390 mg, 1.44 mmol) in THF (5 mL) drop-wise at -10 °C. After the addition was complete, the mixture was stirred for an additional 15 min then concentrated *in vacuo*. The residue obtained was dissolved in hexane-diisopropyl ether (1:1, 15 mL) and the solution was successively washed with 5 % $NaHCO_3$ (10 mL) and H_2O (mL). Drying (Na_2SO_4) and evaporation of solvent *in vacuo* (< 30 °C) gave geranylgeranyl bromide (1.11, 97 %) as a yellow liquid. This bromide (0.97 g, 2.91 mmol) was used without further purification in the conjugate addition reaction mentioned above to give a crude product. Purification by flash column chromatography (SiO_2, petrolum ether-ether, 9:1) gave **48a** (481 mg, 80 %) as a colorless oil: IR ($CHCl_3$ cast) 2948, 2921, 1725, 1642, 1434 cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 5.08 (m, 4 H, 4 x $C{=}C\underline{H}$), 3.74 and 3.73 (2s, 6 H, 2 x $OC\underline{H}_3$), 3.05 (d, 2 H, *J* = 7.1 Hz, $C{=}CHC\underline{H}_2C{=}C$), 2.07-1.96 (m, 12 H, 3 x $(C\underline{H}_2)_2$), 1.95 (s, 3 H, $C\underline{H}_3C{=}C$), 1.67 and 1.65 (2s, 6 H, 2 x $C\underline{H}_3$-chain), 1.59 (s, 9 H, 3 x $C\underline{H}_3$-chain); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.2 and 169.0 (2 x $\underline{C}{=}O$), 138.1, 138.0, 135.2, 134.8, 132.0 and 131.1 (6 x $\underline{C}{=}CH$), 124.4, 124.2, 123.9 and 118.4 (4 x $C{=}\underline{C}H$), 52.1 and 52.0 (2 x $O\underline{C}H_3$), 39.7 ($C{=}CH\underline{C}H_2C{=}C$), 28.9, 26.7, 26.6 and 26.5 (3 x $-(\underline{C}H_2)_2-$), 25.6 ($\underline{C}H_3C{=}CCO$), 17.6, 16.1, 15.9 and 15.1 (5 x $\underline{C}H_3$-chain); HRMS (EI) Calcd for $C_{27}H_{42}O_4$ 430.3083, found 430.3069. Anal. Calcd for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.53; H, 10.07.



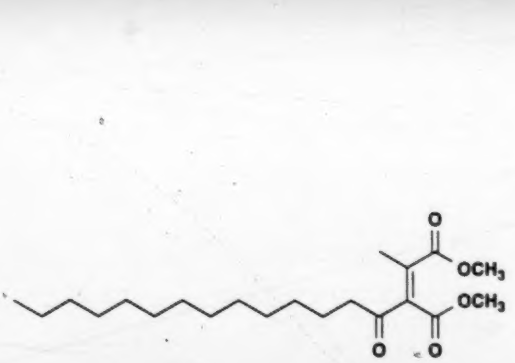
Dimethyl (*E*)-2-(geranylgeranyl)-3-methylbutenedioate (48b). A small amount of the (*E*)-isomer of **48b** (32 mg, 5 %) was also obtained as an oil: IR ($CHCl_3$, cast) 2946, 2919, 1726 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.09 (m, 4 H, 4 x $C{=}C\underline{H}$), 3.77 and 3.74 (2s, 6 H, 2 x $OC\underline{H}_3$), 3.20 (d, 2 H, *J* = 7.2 Hz, $C{=}CHC\underline{H}_2C{=}C$), 2.06-1.94 (m, 12 H, 3 x $(C\underline{H}_2)_2$), 2.01 (s, 3 H, $C\underline{H}_3C{=}C$), 1.67, 1.61 and 1.59 (3s, 15 H, 5 x $C\underline{H}_3$-chain); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.3 and 169.0 (2 x $\underline{C}{=}O$), 138.0, 137.7, 135.1, 134.9, 131.7 and 131.2 (6 x $\underline{C}{=}CH$), 124.4, 124.2, 124.0 and 119.6 (4 x $C{=}\underline{C}H$), 51.9 and 51.7 (2 x $O\underline{C}H_3$), 39.7 ($\underline{C}H_2C{=}C$), 30.2, 26.7 and 26.6 (3 x $-(\underline{C}H_2)_2-$), 23.4 ($\underline{C}H_3C{=}C$), 17.6, 17.5, 16.0 and 15.9 (5 x $\underline{C}H_3$-chain); HRMS (EI) Calcd for $C_{27}H_{42}O_4$ 430.3083, found 430.3071. Anal. Calcd for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.63; H, 10.20.



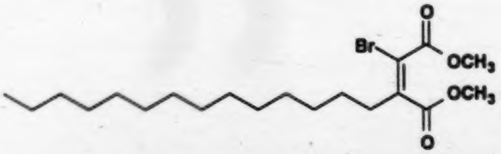


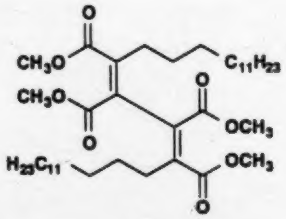
Dimethyl (*Z*)-2-geranyl-3-methylbutenedioate (49a) and dimethyl (*E*)-2-geranyl-3-methylbutenedioate (49b). Following the same procedure as used for the preparation of **40**, CuBr.Me$_2$S (0.82 g, 4.00 mmol), methyl magnesium bromide (1.33 mL of a 3.00 M solution in THF, 4.00 mmol), DMAD (0.497 g, 3.50 mmol) and geranyl bromide (1.52 g, 7.00 mmol) were reacted to give a crude product. Purification by flash column chromatography (SiO$_2$, petroleum ether-ether, 8.5 : 1.5) gave **49a** (877 mg, 85 %) and **49b** (25 mg, 2 %) both as colorless oils. For **49a**: IR (CHCl$_3$ cast) 2950, 1724, 1666, 1434 cm^{-1}; ^{1}H NMR (CDCl$_3$, 360 MHz) δ 5.01-4.97 (m, 2 H, 2 x $C{=}C\underline{H}$), 3.69 and 3.68 (2s, 6 H, 2 x $OC\underline{H}_3$), 3.00 (d, 1 H, J = 7.0 Hz, $C{=}CHC\underline{H}_2C{=}C$), 2.05-1.94 (m, 4 H, $-(C\underline{H}_2)_2-$), 1.90 (s, 3 H, $C\underline{H}_3C{=}CCO$),1.61, 1.59 and 1.53 (3s, 9 H, 3 x $C\underline{H}_3$-chain); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 169.0 and 168.9 (2 x $\underline{C}{=}O$), 137.9, 137.8, 131.9 and 131.3 (4 $\underline{C}{=}C$), 123.8 and 118.4 (2 x $C{=}\underline{C}H$), 51.9 and 51.8 (2 x $O\underline{C}H_3$), 39.4 ($C{=}CH\underline{C}H_2C{=}C$), 28.7 and 26.4 ($-(\underline{C}H_2)_2-$), 25.5 ($\underline{C}H_3C{=}CCO$), 15.9 and 14.9 (3 x $\underline{C}H_3$-chain); HRMS (EI) Calcd for $C_{17}H_{26}O_4$ 294.1831, found 294.1828. Anal. Calcd for $C_{17}H_{26}O_4$: C, 69.36; H, 8.90. Found: C, 69.23; H, 8.91.

For **49b**: IR (CHCl$_3$ cast) 2951, 1725, 1666, 1434 cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 5.09-5.03 (m, 2 H, 2 x $C{=}C\underline{H}$), 3.77 and 3.74 (2s, 6 H, 2 x $OC\underline{H}_3$), 3.20 (d, 1 H, J = 7.2 Hz, $C{=}CHC\underline{H}_2C{=}C$), 2.06-1.95 (m, 4 H, $-(C\underline{H}_2)_2-$), 2.01 (s, 3 H, $C\underline{H}_3C{=}CCO$), 1.67, 1.60 and 1.58 (3s, 9 H, 3 x $C\underline{H}_3$-chain); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 169.3 and 168.1 (2 x $\underline{C}{=}O$), 137.9, 137.7, 131.8 and 131.5 (4 $\underline{C}{=}C$), 124.1 and 119.7 (2 x $C{=}\underline{C}H$), 51.9 and 51.7 (2 x $O\underline{C}H_3$), 39.7 ($C{=}CH\underline{C}H_2C{=}C$), 30.2 and 25.7 ($-(\underline{C}H_2)_2-$), 25.7 ($\underline{C}H_3C{=}CCO$), 17.7 and 16.0 (3 x $\underline{C}H_3$-chain); HRMS (EI) Calcd for $C_{17}H_{26}O_4$ 294.1831, found 294.1836.



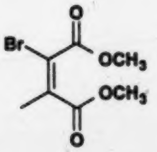
Dimethyl (*Z*)-3-methyl-2-(1'-oxotetradecyl)butenedioate (50). The general procedure for the preparation of **40** was employed using CuBr.Me$_2$S (0.300 g, 1.46 mmol), methylmagnesium bromide (0.49 mL of a 3.00 M solution in ether, 1.47 mmol), DMAD (0.200 g, 1.40 mmol) and myristoyl chloride (0.721 g, 2.92 mmol). Purification in the usual way (SiO$_2$, petrolum ether-ether, 8:2) gave **50** (427 mg, 83 %) as a wax: IR (CHCl$_3$ cast) 2924, 2853, 1736, 1705, 1434 cm^{-1}; ^{1}H NMR (CDCl$_3$, 360 MHz) δ 3.80 and 3.77 (2s, 6 H, 2 x $OC\underline{H}_3$), 2.59 (t, 2 H, J = 7.3 Hz, $CH_2C\underline{H}_2C{=}O$), 2.01 (s, 3 H, $C\underline{H}_3C{=}C$), 1.60 (m, 2 H, $C\underline{H}_2CH_2C{=}O$), 1.42 (br m, 20 H, $(C\underline{H}_2)_{10}$), 0.87 (t, 3 H, J = 6.5 Hz, $C\underline{H}_3$-chain); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 201.2 ($\underline{C}{=}O$, ketone), 168.6 and 164.6 (2 x $\underline{C}{=}O$, ester), 141.5 and 135.7 ($\underline{C}{=}\underline{C}$), 52.6 and 52.5 (2 x $O\underline{C}H_3$), 43.0 ($\underline{C}H_2C{=}O$), 31.9, 29.6, 29.5, 29.4, 29.3, 29.0, 23.3 and 22.6 ($-(\underline{C}H_2)_{11}$), 17.0 ($\underline{C}H_3C{=}C$), 14.1 ($\underline{C}H_3$-chain); HRMS (EI) Calcd for $C_{21}H_{36}O_5$ 368.2563, found 368.2564. Anal. Calcd for $C_{21}H_{36}O_5$: C, 68.45; H, 9.85 Found: C, 68.34; H, 9.95.

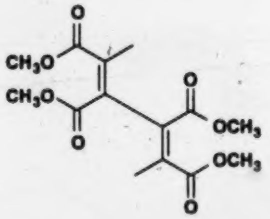




(*E*) Dimethyl 2-bromo-3-tetradecylbutenedioate (51) and (*Z,Z*) dimethyl 3,4-dicarbo-methoxy-2,5-tetradecyl-2,4-hexadiene-1,6-dioate (54). The general procedure for the preparation of **40** was employed. Thus CuBr•Me$_2$S (1.64g, 8.00 mmol), tetradecylmagnesium chloride (8.00 mL of a 1.0 M solution in THF, 8.00 mmol) and freshly distilled DMAD (0.995, 7.00 mmol) were reacted as before. Recrystallized dry NBS (2.49 g, 14.0 mmol) was then added as a solution in THF (20 mL) and the mixture was allowed to warm to - 40 °C and stirred for 1 h before quenching. The usual work up and purification (SiO_2, petrolum ether-ether, 8:2) gave **51** (2.07 g, 70 %) as an oil and **54** (540 mg, 11 %) as a white solid. For **51** IR ($CHCl_3$ cast) 1739, 1616 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.80 and 3.77 (2s, 6 H, 2 x $OC\underline{H}_3$), 2.51 (t, 2 H, *J* = 7.5 Hz, $-CH_2C\underline{H}_2C{=}C$), 1.48 (qn, 2 H, $-C\underline{H}_2CH_2C{=}C$), 1.24 (br s, 22 H, $-(C\underline{H}_2)_{11}$), 0.86 (t, 3 H, *J* = 6.5 Hz, $C\underline{H}_3$-chain); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.8 and 163.9 (2 x $\underline{C}{=}O$), 143.7 and 119.1 ($\underline{C}{=}\underline{C}$), 53.4 and 52.7 (2 x $O\underline{C}H_3$), 34.2, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 26.8 and 22.7 ($-(\underline{C}H_2)_{13}$), 14.1 ($\underline{C}H_3$- chain); HRMS (EI) Calcd. for $C_{20}H_{35}{}^{81}BrO_4$ 420.1698, found 420.1688 and Calcd. for $C_{20}H_{35}{}^{79}BrO_4$ 418.1719, found 418.1712. Anal. Calcd for $C_{20}H_{35}BrO_4$: C, 57.28; H, 8.41. Found: C, 57.51; H, 8.56.

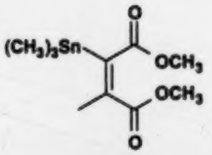
For **54**: IR ($CHCl_3$ cast) 2922, 2852, 1728, 1433 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 3.84 and 3.71 (2s, 12 H, 4 x $OC\underline{H}_3$), 2.26 (m, 4 H, 2 x $C\underline{H}_2C{=}C$), 1.42-1.20 (m, 48 H, 2 x $-(C\underline{H}_2)_{12}-$), 0.88 (t, 6 H, *J* = 6.6 Hz, 2 x $C\underline{H}_3$-chain); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.2 and 165.2 (4 x $\underline{C}{=}O$), 149.0, 125.6 (2 x $\underline{C}{=}\underline{C}$), 52.5 and 52.4 (4 x $O\underline{C}H_3$), 32.3, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 26.7 and 22.7 (2 x $-(\underline{C}H_2)_{11}-$), 14.1 (2 x $\underline{C}H_3$-chains); HRMS (EI) Calcd for $C_{40}H_{70}O_8$ 678.5071, found 678.5080 and for $C_{38}H_{67}O_6$ ($M^+ - C_2H_3O_2$, 100 %) 619.4938, found 619.4936.





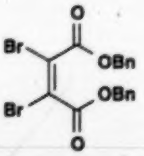
Dimethyl 2-bromo-3-methylbutenedioate (52) and dimethyl 3,4-dicarbomethoxy-2,5-dimethyl-2,4-hexadiene-1,6-dioate (55). The general procedure for the preparation of **40** was employed using CuBr•Me_2S (0.84 g, 4.10 mmol), methylmagnesium bromide (1.37 mL of a 3.00 M in ether, 4.10 mmol), DMAD (0.57 g, 4.00 mmol) and NBS (1.42 g, 8.00 mmol). Trituration of the crude product with petroleum ether-ether (6:4) gave diene **55** (31 mg, 5 %) as a white crystalline solid (see below). Chromatography (SiO_2, petrolum ether-ether, 8.5:1.5) of the concentrated residue gave **54** (711 mg, 75 %) as an oil: IR ($CHCl_3$ cast) 2954, 1739, 1622, 1435 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 3.74 and 3.69 (2s, 6 H, 2 x $OC\underline{H}_3$), 2.05 (s, 3 H, $C\underline{H}_3C{=}C$); MS (CI, NH_3) *m/z* (relative intensity) 256 ($^{81}BrMNH_4^+$, 96%), 254 ($^{79}BrMNH_4^+$, 100%). Anal. Calcd for $C_7H_9BrO_4$: C, 35.47; H, 3.38. Found: C, 35.63; H, 3.51.

For diene **55**: mp 88-89 °C; IR ($CHCl_3$ cast) 1725, 1629 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 3.83 and 3.73 (2s, 12 H, 4 x $OC\underline{H}_3$), 1.95 (s, 6 H, 2 x $C\underline{H}_3C{=}C$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.4 and 165.1 (4 x $\underline{C}{=}O$), 143.5 and 127.1 (2 x $\underline{C}{=}\underline{C}$), 52.6 and 52.5 (4 x $O\underline{C}H_3$), 17.8 (2 x $\underline{C}H_3C{=}C$); HRMS (EI) Calcd for $C_{14}H_{18}O_8$ 314.1002, found 314.0996. Anal. Calcd for $C_{14}H_{18}O_8$: C, 53.50; H, 5.77. Found: C, 53.28; H, 5.57.

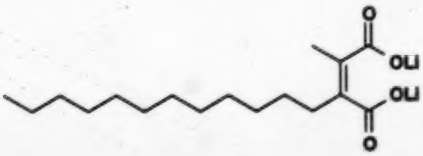


Dimethyl 3-methyl-2-(trimethyltin)butenedioate (53). The general procedure for **40** was employed using $CuBr{\bullet}Me_2S$ (0.82 g, 4.00 mmol), methylmagnesium bromide (1.35 mL of a 3.0 M solution in ether), DMAD (0.500 g, 3.50 mmol) and trimethyltin chloride (8.00 mL of a 1.00 M solution in THF, 8.00 mmol). After the addition of trimethyltin chloride, the reaction mixture was warmed to - 40 °C and stirred for 4 h. Working up the reaction in the usual way gave an orange oil which was triturated with petrolum ether-ether (8:2) to give diene **55** (60 mg, 10 %). Flash column chromatography on the residue (SiO_2, petrolum ether-ether, 9:1) gave **53** (460 mg, 40 %) as a colorless liquid: IR ($CHCl_3$ cast) 1713, 1613, 1433 cm^{-1}; ^1H NMR (CD_2Cl_2, 360 MHz) δ 3.68 and 3.67 (2s, 6 H, 2 x $OC\underline{H}_3$), 2.01 (s, 3 H, $J_{Sn\text{-}H}$ = 4.3 Hz, $C\underline{H}_3C{=}C$), 0.30 (s, 9 H, $J_{Sn\text{-}H}$ = 27.3 Hz, $(C\underline{H}_3)_3Sn$); ^{13}C NMR (CD_2Cl_2, 100 MHz) δ 172.6 and 166.5 (2 x $\underline{C}{=}O$), 149.7

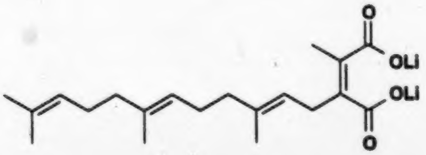
and 139.1 ($\underline{C}$=$\underline{C}$), 52.3 and 51.6 (2 x O$\underline{C}H_3$), 20.8 ($\underline{C}H_3C$=C), -8.0 (($\underline{C}H_3)_3Sn$); MS (FAB, Cleland) *m/z* (relative intensity) 349 (MNa^+, 51%).



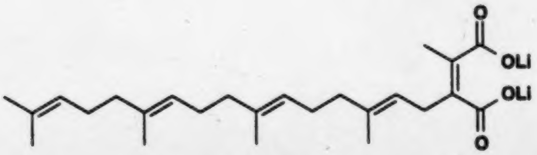
Dibenzyl 2,3-dibromo butenedioate (56). To a suspension of $NaHCO_3$ (1.23 g, 14.6 mmol) in dry DMF (20 mL) was added 2,3-dibromomaleic acid (1.00 g, 3.67 mmol) at room temperature and the mixture was stirred for 10 min. Benzyl bromide (2.49 g, 14.6 mmol) was then added in DMF (5 mL) and the mixture was stirred for 12 h. The reaction mixture was diluted with H_2O (25 mL) and extracted with ether (3 x 40 mL). The combined organic extracts were washed with H_2O (150 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give a red oil. Purification by flash column chromatography (SiO_2, petrolum ether-ether, 9.5:0.5) afforded **56** (473 mg, 29 %) as an oil: IR ($CHCl_3$ cast) 1739, 1585, 1456 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 7.45-7.32 (m, 10 H, 2 x $C_6\underline{H}_5$), 5.13 (s, 4 H, 2 x OC$\underline{H}_2$Ph); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.6 (2 x $\underline{C}$=O), 134.1 and 125.2 (2 x =$\underline{C}$Br + 2 x Ar$\underline{C}$), 128.6, 128.5 and 128.4 (2 x Ar$\underline{C}$H), 68.7 (2 x O$\underline{C}H_2$Ph); MS (CI, NH_3) *m/z* (relative intensity) 472 (MNH_4^+, 100%), 312 (18); Anal. Calcd for $C_{18}H_{10}Br_2O_4$: C, 47.61; H, 3.11. Found: C, 47.68; H, 2.90.



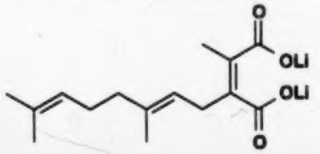
(*Z*)-2-Dodecyl-3-methylbutenedioic acid, di-lithium salt (57). The hydrolysis of ester **46** (50 mg, 0.15 mmol) according to the general procedure given above for the formation of **44** afforded salt **57** (47 mg, 99%) as a white powder: IR (KBr) 2923, 2852, 1593, 1578, 1542, 1433 cm^{-1}; ^1H NMR (CD_3OD, 360 MHz) δ 2.24 (t, 2 H, J = 7.6 Hz, $-CH_2C\underline{H}_2C{=}C$), 1.83 (s, 3 H, $C\underline{H}_3C{=}C$), 1.47 (m, 2 H, $-C\underline{H}_2CH_2C{=}C$), 1.28 (br m, 18 H, $-(C\underline{H}_2)_9$), 0.89 (t, 3 H, J = 6.6 Hz, $C\underline{H}_3$-chain); ^{13}C NMR (CD_3OD, 75 MHz) δ 182.7 and 182.2 (2 x $\underline{C}{=}O$), 141.4 and 134.3 ($\underline{C}{=}\underline{C}$), 34.4, 32.6, 32.2, 32.1, 32.0, 31.9, 31.8, 30.7 and 25.2 ($-(\underline{C}H_2)_{11}$), 17.8 and 16.4 (2 x $\underline{C}H_3$); MS (FAB, Cleland) *m/z* (relative intensity) 311 (MH^+, 16%).



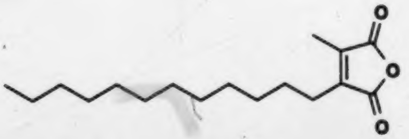
(Z)-2-Farnesyl-3-methylbutenedioic acid, di-lithium salt (58). The hydrolysis of ester **47** (50 mg, 0.14 mmol) gave salt **58** (44 mg, 90%) as a white solid: IR ($CHCl_3$ cast) 2920, 1590, 1543, 1435, 1401 cm^{-1}; ^1H NMR (CD_3OD, 360 MHz) δ 5.23 (m, 1 H, $C{=}C\underline{H}$), 5.08 (m, 2 H, 2 x $C{=}C\underline{H}$), 2.99 (d, 2 H, J = 6.7 Hz, $C{=}CHC\underline{H}_2C{=}C$), 2.11-1.93 (m, 8 H, 2 x $-(C\underline{H}_2)_2-$), 1.83 (s, 3 H, $C\underline{H}_3C{=}CCO$), 1.66 and 1.65 (2s, 6 H, 2 x $C\underline{H}_3$-chain), 1.58 (s, 6 H, 2 x $C\underline{H}_3$-chain); ^{13}C NMR (CD_3OD, 75 MHz) δ 180.5 and 179.5 (2 x $\underline{C}{=}O$), 137.5, 136.2, 135.9, 134.0 and 132.4 (5 x $\underline{C}{=}CH$), 125.5, 125.2 and 123.1 (3 x $C{=}\underline{C}H$), 40.7, 40.6, 30.1, 27.6 and 27.5 (5 x $-\underline{C}H_2-$), 25.9 ($\underline{C}H_3C{=}CCO$), 17.8, 16.4 and 16.1 (4 x $\underline{C}H_3$-chain); MS (CI, NH_3) *m/z* (relative intensity) 364 (MNH_4^+, 6%).



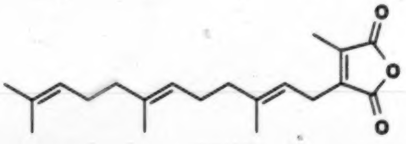
(Z)-2-(Geranylgeranyl)-3-methylbutenedioic acid, di-lithium salt (59). The hydrolysis of ester **48a** (50 mg, 0.12 mmol) gave salt **59** (42 mg, 85%) as a white powder: IR (CH_3OH cast) 2912, 1590, 1540, 1438, 1385 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 5.23 (m, 1 H, $C{=}C\underline{H}$), 5.08 (m, 3 H, 3 x $C{=}C\underline{H}$), 3.00 (d, 2 H, J = 6.7 Hz, C=CHC$\underline{H}_2$C=C), 2.12-1.92 (m, 12 H, 3 x -(C$\underline{H}_2$)$_2$-), 1.84 (s, 3 H, C$\underline{H}_3$C=CCO), 1.67 and 1.66 (2s, 6 H, 2 x C$\underline{H}_3$-chain), 1.58 (s, 9 H, 3 x C$\underline{H}_3$-chain); ^{13}C NMR (CD$_3$OD, 75 MHz) δ 180.0 and 178.9 (2 x $\underline{C}$=O), 137.5, 135.9, 135.8, 134.5 and 132.1 (6 x $\underline{C}$=C), 125.7, 125.5 and 123.4 (4 x C=$\underline{C}$H), 41.0, 40.9, 40.8, 30.4, 27.8 and 27.6 (7 x $\underline{C}$H$_2$), 25.9 ($\underline{C}$H$_3$C=CCO), 17.8, 16.6, 16.4 and 16.1 (5 x $\underline{C}$H$_3$-chain); MS (CI, NH$_3$) *m/z* (relative intensity) 432 (MNH$_4^+$, 21%).



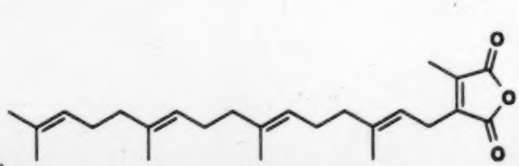
(Z)-2-Geranyl-3-methylbutenedioic acid, di-lithium salt (60). The hydrolysis of ester **49a** (50 mg, 0.17 mmol) gave salt **60** (40 mg, 80%) as a white powder: IR (CHCl$_3$ cast) 2920, 1590, 1543, 1435, 1401 cm^{-1}; ^{1}H NMR (CD$_3$OD, 360 MHz) δ 5.22, 5.08 (2m, 2 H, 2 x C=C$\underline{H}$), 2.99 (d, 2 H, J = 6.6 Hz, C=CHC$\underline{H}_2$C=C), 2.10-1.90 (m, 4 H, -(C$\underline{H}_2$)$_2$-), 1.83 (s, 3 H, C$\underline{H}_3$C=CCO), 1.67 (s, 3 H, C$\underline{H}_3$-chain), 1.61, (s, 6 H, 2 x C$\underline{H}_3$-chain); ^{13}C NMR (CD$_3$OD, 100 MHz) δ 180.5 and 179.5 (2 x $\underline{C}$=O), 137.5, 135.9, 133.8 and 132.3 (4 x $\underline{C}$=C), 125.5 and 124.1 (2 x C=$\underline{C}$H), 33.1, 30.1 and 27.6 (3 x -$\underline{C}$H$_2$-), 25.9 ($\underline{C}$H$_3$C=CCO), 23.6 and 16.5 (3 x $\underline{C}$H$_3$-chain); MS (FAB, Cleland) *m/z* (relative intensity) 279 (MH$^+$, 9%).



(Z)-2-Dodecyl-3-methylbutenedioic acid, anhydride (61). Direct acid work up of lithium salt **57** and ether extraction gave anhydride **61** (42 mg, 99%) as a colorless oil: IR (CH$_2$Cl$_2$ cast) 2925, 2854, 1767, 1466 cm^{-1}; ^{1}H NMR (CD$_3$OD, 360 MHz) δ 2.46 (t, 2 H, J = 7.7 Hz, -CH$_2$C$\underline{H}_2$C=C), 2.03 (s, 3 H, C$\underline{H}_3$C=C), 1.57 (qn, 2 H, -C$\underline{H}_2$CH$_2$C=C), 1.34-1.28 (m, 18 H, -(C$\underline{H}_2$)$_9$-), 0.89 (t, 3 H, J = 6.7 Hz, C$\underline{H}_3$-chain); ^{13}C NMR (CD$_3$OD, 75 MHz) δ 167.8 and 167.5 (2 x $\underline{C}$=O), 145.4 and 141.9 ($\underline{C}$=$\underline{C}$), 33.1, 30.7, 30.6, 30.4, 28.9, 28.5, 25.0 and 23.7 (-($\underline{C}H_2)_{11}$-), 14.4 ($\underline{C}H_3$C=C), 9.3 ($\underline{C}H_3$-chain); HRMS (EI) Calcd for $C_{17}H_{28}O_3$ 280.2039, found 280.2031. Anal. Calcd for $C_{17}H_{28}O_3$: C, 72.82; H, 10.06. Found: C, 72.46; H, 10.24.

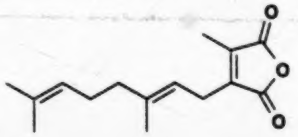


(Z)-2-Farnesyl-3-methylbutenedioic acid, anhydride (62). Acid work up of the solution of salt **58** and isolation according to the general procedure mentioned above gave a residue which was purified by flash column chromatography (SiO_2, petrolum ether-ether, 70:30) to give anhydride **62** (34 mg, 76%) as a colorless oil: IR (CH_2Cl_2 cast) 2941, 1768 cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 5.11 (m, 3 H, 3 x C=C$\underline{H}$), 3.16 (d, 2 H, *J* = 7.3 Hz, C=CHC$\underline{H}_2$C=C), 2.13-1.95 (m, 8 H, 2 x -(C$\underline{H}_2)_2$-), 2.07 (s, 3 H, C$\underline{H}_3$C=CCO), 1.72 and 1.67 (2s, 6 H, 2 x C$\underline{H}_3$-chain), 1.59 (s, 6 H, 2 x C$\underline{H}_3$-chain); ^{13}C NMR (CD_2Cl_2, 100 MHz) δ 166.9 and 166.3 (2 x $\underline{C}$=O), 143.7, 140.5, 140.4, 135.8 and 131.6 (5 x $\underline{C}$=C), 124.6, 124.1 and 116.6 (3 x C=$\underline{C}$H), 40.1, 39.9, 27.1, 26.7 and 23.7 (5 x -$\underline{C}H_2$-), 25.8 ($\underline{C}H_3$C=CCO), 17.7, 16.0 and 9.7 (4 x $\underline{C}H_3$-chain); HRMS (EI) Calcd for $C_{20}H_{28}O_3$ 316.2038, found 316.2033. Anal. Calcd for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.94; H, 9.14.

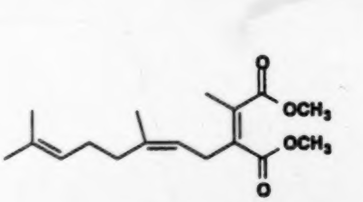


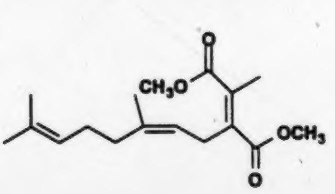
(Z)-2-(Geranylgeranyl)-3-methylbutenedioic acid, anhydride (63). Acid work up and isolation as described before gave a residue which was purified by flash column chromatography (SiO_2, petrolum ether-ether, 80:20) to give anhydride **63** (35 mg, 75%) as a colorless oil: IR (CH_2Cl_2 cast) 2945, 1769 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.11

(m, 4 H, 4 x $C{=}C\underline{H}$), 3.16 (d, 2 H, *J* = 7.3 Hz, $C{=}CHC\underline{H}_2C{=}C$), 2.10-1.95 (m, 12 H, 3 x $-(C\underline{H}_2)_2-$), 2.05 (s, 3 H, $C\underline{H}_3C{=}C$), 1.72 and 1.67 (2s, 6 H, 2 x $C\underline{H}_3$-chain), 1.60 (s, 9 H, 3 x $C\underline{H}_3$-chain); ^{13}C NMR (CD_2Cl_2, 75 MHz) δ 166.9 and 166.3 (2 x $\underline{C}{=}O$), 143.7, 140.5, 140.4, 135.8, 135.3 and 131.6 (6 x $\underline{C}{=}C$), 124.7, 124.5, 124.1 and 116.6 (4 x $C{=}\underline{C}H$), 40.1, 40.0, 39.9, 27.2, 27.0, 26.7 and 23.8 (7 x $-\underline{C}H_2-$), 25.8 ($\underline{C}H_3C{=}CCO$), 17.7, 16.5, 16.1 and 9.7 (5 x $\underline{C}H_3$-chain); HRMS (EI) Calcd for $C_{25}H_{36}O_3$ 384.2665, found 384.2660. Anal. Calcd for $C_{25}H_{36}O_3$: C, 78.08; H, 9.44. Found: C, 78.21; H, 9.31.



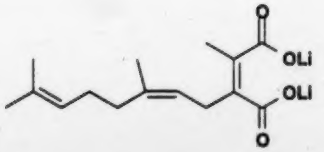
(*Z*)-2-Geranyl-3-methylbutenedioic acid, anhydride (64). Acid work up and isolation as before gave a residue which was purified by flash column chromatography (SiO_2, petrolum ether-ether, 90:10) to give anhydride **64** (35 mg, 83%) as a colorless oil: IR ($CHCl_3$ cast) 2967, 1767, 1673 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 5.11-5.04 (m, 2 H, 2 x $C{=}C\underline{H}$), 3.16 (d, 2 H, *J* = 7.2 Hz, $C{=}CHC\underline{H}_2C{=}C$), 2.07 (s, 3 H, $C\underline{H}_3C{=}CCO$), 2.04-2.00 (m, 4 H, $-(C\underline{H}_2)_2-$), 1.70, 1.65 and 1.58 (3s, 9 H, 3 x $C\underline{H}_3$-chain); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.4 and 165.7 (2 x $\underline{C}{=}O$), 143.3, 140.0, 139.9 and 131.8 (4 x $\underline{C}{=}C$), 123.6 and 116.1 (2 x $C{=}\underline{C}H$), 39.5 ($C{=}CH\underline{C}H_2C{=}C$), 26.3 and 23.4 ($-(\underline{C}H_2)_2-$), 25.6 ($\underline{C}H_3C{=}CCO$), 16.3 and 9.5 (3 x $\underline{C}H_3$-chain); HRMS (EI) Calcd for $C_{15}H_{20}O_3$ 248.1413, found 248.1402. Anal. Calcd for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.21; H, 7.99.



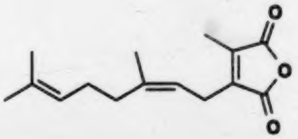


Dimethyl (*Z*)-2-nerolyl-3-methylbutenedioate (65a) and dimethyl (*E*)-2-nerolyl-3-methylbutenedioate (65b). The general procedure for preparing **40** was followed. Thus, CuBr•Me_2S (0.821 g, 4.00 mmol), methylmagnesium bromide (1.33 mL of a 3.0 M solution in THF, 4.00 mmol) and DMAD (0.497 g, 3.50 mmol) were allowed to react. Concurrently, neryl bromide was being prepared by adding a solution of phosphorous tribromide (1.02 g, 3.77 mmol) in THF (5 mL) to nerol (1.39 g, 9.00 mmol) in THF (10 mL) at -10 °C. Isolating the product in the same way as for the preparation of geranylgeranyl bromide (see compound **48a**) gave the crude nerolyl bromide **66** (1.66 g, 85 %). This product (1.55 g, 7.14 mmol) was used without further purification to trap the conjugate addition adduct made above to give crude product. Purification by flash column chromatography (SiO_2, petroleum ether-ether 8.5 : 1.5) gave **65a** (751 mg, 73 %) and **65b** (17 mg, 1.7 %) both as colorless oils. For **65a**: IR ($CHCl_3$ cast) 2950, 1724, 1663, 1434 cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 5.07-4.96 (m, 2H, 2 x C=C$\underline{\text{H}}$), 3.70 and 3.69 (2s, 6 H, 2 x OC$\underline{\text{H}}_3$), 3.01 (d, 2 H, J = 6.9 Hz, C=CHC$\underline{\text{H}}_2$C=C), 2.03 (m, 4 H, -(C$\underline{\text{H}}_2$)$_2$-), 1.91 (s, 3 H, C$\underline{\text{H}}_3$C=CCO), 1.65, 1.64 and 1.56 (3s, 9 H, 3 x C$\underline{\text{H}}_3$-chain); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.0 and 168.9 (2 x $\underline{\text{C}}$=O), 137.9, 137.8, 132.0 and 131.6 (4 x $\underline{\text{C}}$=C), 123.8 and 119.0 (2 x C=$\underline{\text{C}}$H), 52.0 and 51.9 (2 x O$\underline{\text{C}}$H$_3$), 31.9 (C=CH$\underline{\text{C}}$H$_2$C=C), 28.5, 26.2 (-($\underline{\text{C}}$H$_2$)$_2$-), 25.4 ($\underline{\text{C}}$H$_3$C=CCO), 23.1, 17.5 and 15.0 (3 x $\underline{\text{C}}$H$_3$-chain); HRMS (EI) Calcd for $C_{17}H_{26}O_4$ 294.1831, found 294.1833. Anal. Calcd for $C_{17}H_{26}O_4$: C, 69.36; H, 8.90. Found: C, 69.33; H, 8.87.

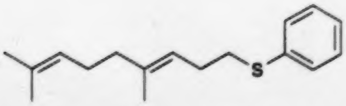
For **65b**: IR ($CHCl_3$ cast) 2952, 2927, 1724, 1647, 1434 cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 5.10-5.00 (m, 2 H, 2 x C=C$\underline{\text{H}}$), 3.77 and 3.75 (2s, 6 H, 2 x OC$\underline{\text{H}}_3$), 3.20 (d, 2 H, J = 7.0 Hz, C=CHC$\underline{\text{H}}_2$C=C), 2.10-1.98 (m, 4 H, -(C$\underline{\text{H}}_2$)$_2$-), 2.02 (s, 3 H, C$\underline{\text{H}}_3$C=CCO), 1.68 (s, 6 H, 2 x C$\underline{\text{H}}_3$-chain), 1.61 (s, 3 H, C$\underline{\text{H}}_3$-chain); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.3 and 168.1 (2 x $\underline{\text{C}}$=O), 137.9, 137.7, 131.8 and 131.5 (4 x $\underline{\text{C}}$=C), 124.1 and 119.7 (2 x C=$\underline{\text{C}}$H), 51.9 and 51.7 (2 x O$\underline{\text{C}}$H$_3$), 39.7 (C=CH$\underline{\text{C}}$H$_2$C=C), 30.2 and 25.7 (-($\underline{\text{C}}$H$_2$)$_2$-), 25.7 ($\underline{\text{C}}$H$_3$C=CCO), 17.7 and 16.0 (3 x $\underline{\text{C}}$H$_3$-chain); HRMS (EI) Calcd for $C_{17}H_{26}O_4$ 294.1831, found 294.1823.



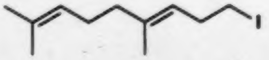
(Z)-2-Nerolyl-3-methylbutenedioic acid, di-lithium salt (67). The hydrolysis of ester **65a** (50 mg, 0.17 mmol) gave salt **67** (43 mg, 90%) as a white powder: IR ($CHCl_3$ cast) 2920, 1590, 1543, 1435, 1401 cm^{-1}; ^1H NMR (CD_3OD, 360 MHz) δ 5.22 and 5.15 (2m, 2 H, 2 x $C{=}C\underline{H}$), 2.99 (d, 2 H, *J* = 6.6 Hz, $C{=}CHC\underline{H_2}C{=}C$), 2.10 (m, 4 H, $-(C\underline{H_2})_2-$), 1.83 (s, 3 H, $C\underline{H_3}C{=}CCO$), 1.67 (s, 6 H, 2 x $C\underline{H_3}$-chain) 1.61, (s, 3 H, $C\underline{H_3}$-chain); ^{13}C NMR (CD_3OD, 100 MHz) δ 180.5 and 179.5 (2 x $\underline{C}{=}O$), 137.5, 135.9, 133.8 and 132.3 (4 x $\underline{C}{=}C$), 125.5 and 124.1 (2 x $C{=}\underline{C}H$), 33.1, 30.1 and 27.6 (3 x $-\underline{C}H_2-$), 25.9 ($\underline{C}H_3C{=}CCO$), 23.6, 17.7 and 16.5 (3 x $\underline{C}H_3$-chain); MS (FAB, Cleland) *m/z* (relative intensity) 279 (MH^+, 28%).



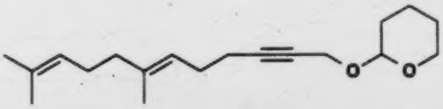
(Z)-2-Nerolyl-3-methylbutenedioic acid, anhydride (68). Acid work up and isolation as before gave a residue which was purified by flash column chromatography (SiO_2, petrolum ether-ether, 90:10) to give anhydride **68** (33 mg, 78%) as a colorless oil: IR ($CHCl_3$ cast) 2961, 1764, 1667 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 5.14-5.04 (m, 2 H, 2 x $C{=}C\underline{H}$), 3.16 (d, 2 H, *J* = 7.4 Hz, $C{=}CHC\underline{H_2}C{=}C$), 2.13-2.06 (m, 4 H, $-(C\underline{H_2})_2-$), 2.07 (s, 3 H, $C\underline{H_3}C{=}CCO$), 1.72, 1.67 and 1.61 (3s, 9 H, 3 x $C\underline{H_3}$-chain); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.4 and 165.7 (2 x $\underline{C}{=}O$), 143.3, 140.0, 139.9 and 132.2 (4 x $\underline{C}{=}C$), 123.5 and 116.8 (2 x $C{=}\underline{C}H$), 31.9 ($C{=}CH\underline{C}H_2C{=}C$), 26.1 and 23.2 ($-(\underline{C}H_2)_2-$), 25.7 ($\underline{C}H_3C{=}CCO$), 17.6 and 9.5 (3 x $\underline{C}H_3$-chain); HRMS (EI) Calcd for $C_{15}H_{20}O_3$ 248.1413, found 248.1414. Anal. Calcd for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.46; H, 7.97.



Homogeranyl phenylsulfide (69). To a solution of Dabco (4.08 g, 36.4 mmol) and thioanisole (4.52 g, 36.4 mmol) in dry THF (100 mL) at 0 °C was added *n*-BuLi (15.6 mL of a 2.5 M solution in hexanes, 38.9 mmol) over 20 min. After the addition was complete, the mixture was allowed to warm to room temperature. To the resulting phenylthiomethyllithium solution was added copper iodide (7.63 g, 40.0 mmol) at -50 °C. After 2 h geranyl bromide (7.19 g, 33.1 mmol) was added drop-wise at -20 °C and the mixture was stirred for 11 h then warmed to room temperature slowly. Water (100 mL) was added and the mixture was extracted with pentane (3 x 150 mL) The combined organic extracts were washed with brine (200 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give **69**[206] (7.8 g, 90 %) as a yellow oil. This material was used in the next step without any further purification: IR ($CHCl_3$ cast) 2965, 2917, 2853, 1585, 1480, 1438 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 7.34-7.16 (m, 5 H, $C_6\underline{H}_5$), 5.22 and 5.13 (2m, 2 H, 2 x $C{=}C\underline{H}$), 2.98 (dt, 2 H, *J* = 7.4, 7.3 Hz, $-C\underline{H}_2S-$), 2.36 (q, 2 H, *J* = 7.5 Hz, $-C\underline{H}_2CH_2SPh$), 2.12-2.00 (m, 4 H, $-(C\underline{H}_2)_2-$), 1.71, 1.63 and 1.61 (3s, 9 H, 3 x $C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 137.1, 136.9 and 131.4 (2 x $\underline{C}{=}CH$ + Ar$\underline{C}$), 129.0, 128.7, 125.7, 124.2 and 122.0 (2 x $C{=}\underline{C}H$ + 5 x Ar$\underline{C}H$), 39.6 ($\underline{C}H_2SPh$), 33.6 ($-\underline{C}H_2CH_2SPh$), 27.8 and 26.6, ($-(\underline{C}H_2)_2-$), 25.7, 17.6 and 16.1 (3 x $\underline{C}H_3$); HRMS (EI) Calcd for $C_{17}H_{24}S$ 260.1599, found 260.1596.

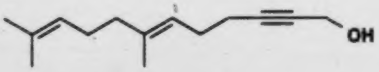


Homogeranyl iodide (70). To sulfide **69** (7.33 g, 28.0 mmol) in dry DMF (48 mL) was added successively freshly distilled methyl iodide (53.7 g, 379 mmol), sodium iodide (11.5 g, 76.6 mmol), calcium carbonate (183 mg, 1.83 mmol) and two drops of mercury. The mixture was then heated at 67 °C for 20 h. After cooling, the reaction mixture was poured into water (200 mL) stirred for 20 min and extracted with pentane (5 x 250 mL). The organic extracts were washed with brine (250 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give 8.5 g of crude product. Purification by flash column chromatography (SiO_2, petrolum ether) gave 5.64 g (77 %) of the product **70**[206] as a colorless liquid which quickly turns light orange upon standing in the air: IR ($CHCl_3$ cast) 2954, 2920, 1581, 1437 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 5.11 (m, 2 H, 2 x C=C$\underline{H}$), 3.11 (t, 2 H, J = 7.4 Hz, -C$\underline{H}_2$I), 2.58 (q, 2 H, J = 7.3 Hz, -C$\underline{H}_2$CH$_2$I), 2.12-1.97 (m, 4 H, -(C$\underline{H}_2$)$_2$-), 1.68 and 1.61 (2s, 9 H, 3 x C$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.0 and 131.4 (2 x $\underline{C}$=CH), 124.0 and 123.0 (2 x C=$\underline{C}$H), 109.8 ($\underline{C}$H$_2$I), 39.6 (-$\underline{C}$H$_2$CH$_2$I), 32.4 and 26.4 (-($\underline{C}$H$_2$)$_2$-), 25.7, 17.7 and 16.2 (3 x $\underline{C}$H$_3$); HRMS (EI) Calcd for $C_{11}H_{19}I$ 278.0553, found 278.0520 and for $C_{11}H_{18}I$ (M^+ -H) 277.0453, found 277.0453.

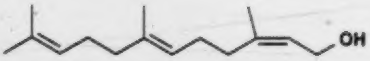


***O*-(Tetrahydropyranyl)-3-homogeranyl propargyl alcohol (71).** To a solution of tetrahydro-2-(2-propynyloxy)-2*H*-pyran (1.44 g, 10.3 mmol) in dry THF (24 mL), and dry HMPA (5.52 g, 30.8 mmol) at -78 °C was added *n*-BuLi (7.06 mL of a 1.6 M solution in hexanes, 11.3 mmol). After the addition was complete, the reaction mixture was allowed to warm to 0 °C and held at this temperature for 20 min before it was cooled back to -78 °C. A solution of iodide **70** (3.00 g, 10.8 mmol) in THF (5 mL) was added and the mixture was stirred at 0 °C for 12 h and at room temperature for 1h. Water (30 mL) was added and the mixture was extracted with ether (5 x 50 mL). The combined organic extracts were washed with water (100 mL) and brine (100 mL) and dried (Na_2SO_4). Concentration *in vacuo* gave the crude product which was purified by flash column chromatography (SiO_2, petroleum ether-ether, 94:6) to give **71** (2.0 g, 70 %) as a clear colorless oil: IR (CH_2Cl_2 cast) 2939, 2869, 1441, 1023 cm^{-1}; ^1H NMR ($CDCl_3$, 300

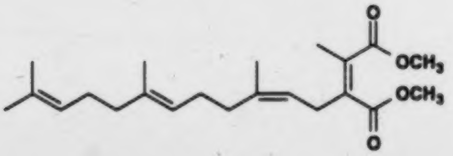
MHz) δ 5.21-5.03 (m, 2 H, 2 x C=C$\underline{H}$), 4.78 (t, 1 H, *J* = 3.2 Hz, OC$\underline{H}$O), 4.28-4.13 (br q, 2 H, *J* = 15.2 Hz, alkyne-CH_2O-), 3.83 (m, 1 H, -$CH_2C\underline{H}HO$ of THP), 3.51 (m, 1 H, -$CH_2CH\underline{H}O$ of THP), 2.21 (m, 4 H, C=CH($C\underline{H}_2$)$_2$-alkyne), 2.14-1.93 (m, 4 H, C=CH-($C\underline{H}_2$)$_2$-C=CH), 1.85-1.48 (m, 6 H, ($C\underline{H}_2$)$_3$ of THP), 1.68, 1.61, 1.60 (3s, 9 H, 3 x $C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 136.5, 131.3 (2 x $\underline{C}$=CH), 124.2, 122.6 (2 x C=$\underline{C}$H), 96.6 (O$\underline{C}$HO), 86.5, 75.7 (2 alkyne $\underline{C}$), 61.9 (alkyne-$\underline{C}H_2O$), 54.6 (-$CH_2\underline{C}H_2O$ of THP), 39.6, 30.3, 27.3, 26.6, 25.4, 19.3, 19.1 (7 x $\underline{C}H_2$), 25.6, 17.6, 16.1 (3 x CH_3); HRMS (EI) Calcd for $C_{19}H_{30}O_2$ 290.4460, found 290.4448. Anal. Calcd for $C_{19}H_{30}O_2$: C, 78.57; H, 10.41. Found: C, 78.26; H, 10.48.



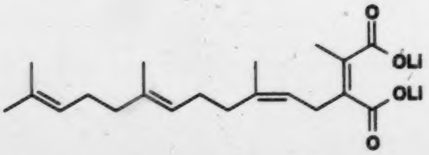
3-Homogeranyl propargyl alcohol (72). To a solution of ether **71** (1.94 g, 6.67 mmol) in methanol (40 mL) was added *p*-toluenesulphonic acid monohydrate (254 mg, 1.33 mmol) and the mixture was stirred at room temperature and monitored by tlc for the consumption of starting material. After 2.5 h the reaction mixture was poured into 5% $NaHCO_3$ (10 mL) and partitioned between H_2O and ethyl acetate. The aqueous layer was extracted with ethyl acetate (3 x 25 mL). The combined organic extracts were washed with H_2O and brine and then dried over Na_2SO_4. Evaporation of the solvent *in vacuo* followed by flash column chromatography (SiO_2, petrolum ether-ether, 4:1) gave the known compound **72** (1.34 g, 97 %) as a colorless oil: IR ($CHCl_3$ cast) 3550-3300 (br), 2924, 2916, 2223, 1650, 1444 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.15-5.02 (m, 2 H, 2 x C=C$\underline{H}$), 4.20 (s, 2 H, -$C\underline{H}_2OH$), 2.32 (s, 1 H, O$\underline{H}$), 2.19 (m, 4 H, C=CH-($C\underline{H}_2$)$_2$-alkyne), 2.07-1.91 (m, 4 H, C=CH-($C\underline{H}_2$)$_2$-C=CH), 1.65, 1.58 and 1.57 (3s, 9 H, 3 x $C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 136.6 and 131.3 (2 x $\underline{C}$=CH), 124.2 and 122.5 (2 x C=$\underline{C}$H), 86.1 and 78.3 (2 x alkyne-$\underline{C}$), 51.1 (-$\underline{C}H_2OH$), 39.6, 27.2, 26.4 and 19.2 (4 x $\underline{C}H_2$), 25.8, 17.6 and 16.0 (3 x $\underline{C}H_3$); HRMS (EI) Calcd for $C_{14}H_{22}O$ 206.1671, found 206.1660.



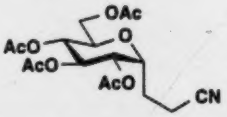
2-(*Z*)-6-(*E*)-Farnesol (73). To a solution of isobutylmagnesium chloride (3.13 mL of a 2.0 M solution in ether, 6.26 mmol) was added $(\pi^5\text{-}C_5H_5)_2TiCl_2$ (67.2 mg, 0.270 mmol) at 0 °C and the mixture was stirred for 5 min. Acetylenic alcohol **72** (562 mg, 2.72 mmol) was added in ether (3 mL) and the reaction mixture was allowed to warm to room temperature and stirred for 2.5 h. After removal of ether *in vacuo*, the residue was dissolved in THF (10 mL) and treated with freshly distilled methyl iodide (992 mg, 6.99 mmol) at 0 °C for 15 min and at room temperature for 3.5 h and then poured into ice-cold H_2O (40 mL). The mixture was repeatedly extracted with ethyl acetate (6 x 60 mL) and the combined organic extracts were washed with H_2O (100 mL), brine (100 mL) and dried over Na_2SO_4. Evaporation of solvent *in vacuo* gave an orange liquid which was purified by flash column chromatography (SiO_2, petrolum ether-ether, gradient, 0 to 20% ether) to afford **73** (401 mg, 66 %) as a colorless oil: IR ($CHCl_3$ cast) 3540-3265 (br), 2965, 2927, 1651, 1444 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.44 (m, 1 H, C=C$\underline{H}$), 5.15-5.07 (m, 2 H, 2 x C=C$\underline{H}$), 4.10 (dd, 2 H, *J* = 0.9, 0.9 Hz, C$\underline{H}_2$OH), 2.11 (s, 3 H, C$\underline{H}_3$C=C), 2.12-1.95 (m, 8 H, 2 x -(C$\underline{H}_2$)$_2$-), 1.75 , 1.68 and 1.59 (3s, 9 H, 3 x C$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 140.0, 136.0 and 131.4 (3 x $\underline{C}$=CH), 124.3, 124.2 and 123.6 (3 x C=$\underline{C}$H), 59.0 ($\underline{C}H_2O$), 39.7, 32.0, 26.6, 26.5, 25.7, 23.4, 17.7 and 16.0 (2 x ($\underline{C}H_2)_2$ and 4 x $\underline{C}H_3C$=C); HRMS (EI) Calcd for $C_{15}H_{26}O$ 222.1984, found 222.1978.

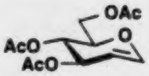


Dimethyl (*Z*)-2-(2'-(*Z*)-6'-(*E*)-farnesyl)-3-methylbutenedioate (75). The general protocol given for the preparation of **40** was followed. Thus CuBr•Me_2S (124 mg, 0.60 mmol), methyl magnesium bromide (0.20 mL of a 3.0 M solution in ether, 0.60 mmol) and DMAD (71.0 mg, 0.50 mmol) were reacted in the usual way. Concurrently 2-(*Z*)-6,10-(*E,E*)-farnesyl bromide was being prepared according to the method presented above for the preparation of geranylgeranyl bromide (see compound **48a**). Reaction between phosphorous tribromide (144 mg, 0.530 mmol) and alcohol **73** (280 mg, 1.26 mmol) gave, after the usual work up, 2-(*Z*)-6,10-(*E,E*)-farnesyl bromide **74** (283 mg, 94 %). This product (250 mg, 0.876 mmol) was used to trap the conjugate addition adduct made above to give the crude product. Purification by flash column chromatography (SiO_2, petroleum ether-ether 85:15) gave **75** (130 mg, 72 %) as an oil: IR (neat film) 2949, 2932, 1724, 1644, 1434 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 5.15-5.00 (m, 3 H, 3 x $C{=}C\underline{H}$), 3.75, 3.74 (2s, 6 H, 2 x $OC\underline{H}_3$), 3.05 (d, 2 H, *J* = 7.0 Hz, $C{=}CHC\underline{H}_2C{=}C$), 2.08-1.94 (m, 8 H, 2 x $(C\underline{H}_2)_2$), 1.96 (s, 3 H, $C\underline{H}_3C{=}CCO$) 1.70 and 1.67 (2s, 6 H, 2 x $C\underline{H}_3$-chain), 1.60 (s, 6 H, 2 x $C\underline{H}_3$-chain); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.2 and 169.0 (2 x $\underline{C}{=}O$), 138.2, 138.1, 135.6, 132.3 and 131.4 (5 x $\underline{C}{=}C$), 124.3, 123.9 and 119.1 (3 x $C{=}\underline{C}H$), 52.2 and 52.1 (2 x $O\underline{C}H_3$), 39.8 ($C{=}CH\underline{C}H_2C{=}C$), 32.1, 28.8, 26.8 and 26.4 (2 x $(\underline{C}H_2)_2$), 25.7, 23.4, 17.7, 16.0 and 15.2 (5 x $\underline{C}H_3$); HRMS (EI) Calcd. for $C_{15}H_{20}O_4$ (M^+ - C_7H_{14}) 264.1361, found 264.1349.



(*Z*)-2-(2'-(*Z*)-6'-(*E*)-Farnesyl)-3-methylbutenedioic acid, di-lithium salt (76). The hydrolysis of ester **75** (50 mg, 0.14 mmol) according to the standard procedure described above for the formation of **44** gave salt **76** (35 mg, 67%). IR ($CHCl_3$ cast) 2920, 1590, 1543, 1435, 1401 cm^{-1}; ^1H NMR (CD_3OD, 360 MHz) δ 5.25–5.07 (m, 3 H, 3 x $C{=}C\underline{H}$), 2.98 (d, 2 H, *J* = 6.7 Hz, $C{=}CHC\underline{H}_2C{=}C$), 2.08-1.92 (m, 8 H, 2 x -$(CH_2)_2$), 1.90 (s, 3 H, $C\underline{H}_3C{=}CCO$), 1.68 and 1.65 (2s, 6 H, 2 x $C\underline{H}_3$-chain), 1.60 (s, 6 H, 2 x $C\underline{H}_3$-chain); ^{13}C NMR (CD_3OD, 100 MHz) δ 180.4 and 179.2 (2 x $\underline{C}{=}O$), 137.3, 137.1, 136.1, 135.9, 133.9, 125.6, 125.5 and 124.1 (8 x =$\underline{C}$), 40.9, 33.1, 30.2, 27.8 and 27.5 (5 x -$\underline{C}H_2$-), 25.9 ($\underline{C}H_3C{=}CCO$), 23.7, 17.8, 16.5 and 16.1 (4 x $\underline{C}H_3$-chain); MS (CI, NH_3) *m/z* (relative intensity) 364 (MNH_4^+, 9 %).



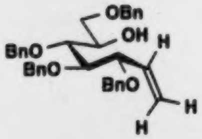


1-Deoxy-2,3,4,6-tetra-*O*-acetyl-1-(2'-cyanoethyl)-α-D-glucopyranose (78) and 1,2-dideoxy-3,4,5-tri-*O*-acetyl glucal (79). A mixture of Vitamin B_{12a} (200 mg, 0.145 mmol); NH_4Cl (780 mg, 14.6 mmol) and activated zinc powder (4.70 g, 71.9 mmol) in DMF (50 mL) was degassed and then stirred under argon until it became dark green (1.5 h). Freshly distilled acrylonitrile (770 mg, 14.5 mmol) was then added, followed by acetobromoglucose (2.00 g, 4.86 mmol) and the mixture was stirred for 12 h at room temperature. The thick mixture was filtered and the solid was washed with CH_2Cl_2. The filtrate was concentrated *in vacuo* to give a brown residue which was dissolved in CH_2Cl_2 (125 mL) and washed with 2.5 % aqueous NH_3 (100 mL) and brine (2 x 50 mL). Drying (Na_2SO_4) and evaporation *in vacuo* gave 1.65 g of a yellow oil. Purification by flash column chromatography (SiO_2, CH_2Cl_2-ether, 10:1) gave **78** (790 mg, 42 %) as a white solid along with **79** (447 mg, 41 %) as a colorless oil.

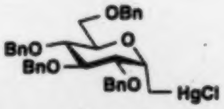
For **78**: mp. 123-124 °C (lit.[233] mp 121-122 °C); IR ($CHCl_3$ cast) 2247 , 1747 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 5.24 (t, 1 H, J = 8.3 Hz, $\underline{H}$-3), 5.09 (dd, 1 H, J = 8.5, 5.2 Hz, $\underline{H}$-2), 4.96 (t, 1 H, J = 8.3 Hz, $\underline{H}$-4), 4.31 (dd, 1 H, J = 12.3, 5.8 Hz, $\underline{H}$-6a), 4.20 (m, 1 H , $\underline{H}$-1), 4.10 (dd, 1 H, J = 12.2, 2.8 Hz, $\underline{H}$-6b), 3.87 (ddd, 1 H, J = 8.5, 5.8, 2.9 Hz, $\underline{H}$-5), 2.45 (m, 2 H, -$C\underline{H}_2CN$), 2.20-2.05 (m, 1 H, $CC\underline{H}HCH_2CN$), 2.10 and 2.07 (2s, 6 H, 2 x OAc), 2.04 (s, 6 H, 2 x OAc), 1.92-1.83 (m, 1 H, $CCH\underline{H}CH_2CN$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.5, 169.7 and 169.4 (4 x $\underline{C}{=}O$), 118.7 ($\underline{C}N$), 70.7, 69.9, 69.7, 69.4 and 68.1 (5 $\underline{C}H$), 61.8 ($\underline{C}H_2O$), 22.7 ($\underline{C}H_2$), 20.7 and 20.6 (4 x $\underline{C}H_3CO$), 13.3 ($\underline{C}H_2$);

HRMS (EI) calcd for $C_{17}H_{24}O_9N$ 386.1451, found 386.1441; Anal. Calcd for $C_{17}H_{24}O_9N$: C, 52.98; H, 6.02; N, 3.63. Found: C, 52.76; H, 5.99; N, 3.47.

For **79**: IR ($CHCl_3$ cast) 2960, 1743, 1650, 1370 cm^{-1}; ^{1}H NMR ($CHCl_3$, 360 MHz) δ 6.43 (dd, 1 H, *J* = 6.2, 1.1 Hz, H-1), 5.32-5.29 (m, 1 H, H-3), 5.19 (dd, 1 H, *J* = 7.5, 1.8 Hz, H-2), 4.81 (dd, 1 H, *J* = 6.2, 3.3 Hz, H-6a), 4.36 (dd, 1 H, *J* = 12.0, 5.7 Hz, H-4), 4.25-4.20 (m, 1 H, H-5), 4.16 (dd, 1 H, *J* = 12.0, 3.1 Hz, H-6b), 2.07, 2.05, 2.01 (3s, 9 H, 3 x CH_3CO); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.4, 170.3 and169.4 (3 x C=O), 145.5 (C-1), 98.9 (C-2), 73.9 (C-3), 67.3 and 67.1 (C-4 and C-5), 61.3 (C-6), 20.9, 20.7 and 20.6 (3 x CH_3CO); HRMS (ES) calcd for $C_{12}H_{16}O_7Na$ 295.0794, found 295.0789. Anal. Calcd for $C_{12}H_{16}O_7$: C, 52.94; H, 5.92. Found: C, 53.23; H, 5.90.

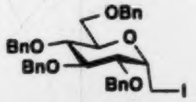


3,4,5,7-Tetra-*O*-benzyl-1,2-dideoxy-D-*gluco*-hept-1-enitol (81). Dr. Lei Qiao's procedure was followed.[236] *n*-Butyllithium (19.3 mL of a 1.6 M solution in hexanes, 30.8 mmol) was added dropwise to a stirred suspension of methyltriphenylphosphonium bromide (11.0 g, 30.8 mmol) in dry DME (50 mL) at -78 °C under an argon atmosphere. After completion of the addition, the reaction mixture was allowed to warm to room temperature and stirring was continued for 30 min to give a suspension of the ylide. To a suspension of 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (6.00 g, 11.1 mmol) in dry DME (46 mL) was added *n*-butyllithium (6.94 mL of a 1.6 M solution in hexanes, 11.1 mmol) over a period of 5 min at -78 °C under argon. The cooling bath was removed and the mixture was stirred at room temperature for 20 min to give a clear solution. The ylide prepared above was added to this latter solution rapidly *via* cannula and the resulting suspension was heated at 45 °C for 2 h. TLC analysis indicated complete consumption of carbohydrate starting material. Acetone (60 mL) was added to quench the reaction and the resulting solution was stirred at 22 °C for an additional 2 h. The solvents were evaporated *in vacuo* and the yellowish residue was suspended in brine and extracted with CH_2Cl_2 (3 x 50 mL). The combined extracts were dried ($MgSO_4$) and concentrated *in vacuo* to yield a yellowish syrup. Purification by flash chromatography (SiO_2, petroleum ether-ethyl acetate, gradient, 10:1 to 3:1) afforded olefin **81** (5.1 g, 86 %) as a waxy solid: IR (CH_2Cl_2 cast) 3470, 3061, 3032, 2862, 1450, 1354 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.40-7.28 (m, 20 H, ArH), 5.89 (ddd, 1 H, *J* = 17.0, 10.0, 7.5 Hz, H-2), 5.31-5.19 (m, 2 H, H-1a, H-1b), 4.86 (d, 1 H, *J* = 11.3 Hz, PhCHHO), 4.68 (d, 1 H, *J* = 11.3 Hz, PhCHHO), 4.62 (d, 1 H, *J* = 11.3 Hz, PhCHHO), 4.61-4.52 (2 d, 2 H, *J* = 11.3 Hz, 2 x $PhCH_2O$), 4.50-4.46 (ABq, 2 H, *J* = 11.3 Hz, $PhCH_2O$), 4.43 (d, 1 H, *J* = 11.3 Hz, PhCHHO), 4.21 (dd, 1 H, *J* = 7.5, 5.0 Hz, H-3), 4.05-3.99 (m, 1 H, H-6), 3.77-3.71 (m, 2 H, H-4, H-5), 3.64-3.50 (m, 2 H, H-7a, H-7b); ^{13}C NMR (CD_2Cl_2, 75 MHz) δ 139.1, 139.0, 138.6, 136.0, 128.8, 128.6, 128.4, 128.2, 128.1, 127.8, 119.3, 82.1, 79.1, 75.2, 73.8, 73.7, 71.7, 71.2, 70.9; MS (CI, NH_3) *m/z* (relative intensity) 556 (MNH_4^+, 34%), 539 (MH^+, 11), 431 (9), 91 (100).

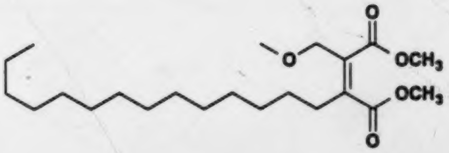


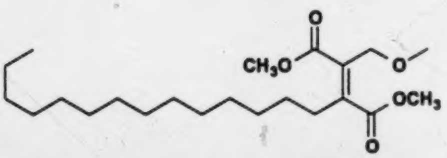
(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)methylmercuric chloride (83). The literature procedure[237a] was modified as follows: Mercuric acetate (4.95 g, 15.5 mmol) was added to a stirred solution of olefin **81** (4.92 g, 9.13 mmol) in dry THF (120 mL) under an argon atmosphere. The resulting solution was stirred at room temperature for 20 h and then a solution of potassium chloride (8.9 g, 11.9 mmol) in H_2O (50 mL) was added. The resultant mixture was stirred at room temperature for an additional 4 h and then the organic layer was separated. The aqueous solution was extracted with CH_2Cl_2 (3 x 60 mL) and the combined organic extracts were washed with brine (100 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification of the residue by

flash chromatography (SiO_2, petroleum ether-ethyl acetate-methanol, 25:5:1) afforded **83** (5.82 g, 82 %) as a syrup: IR (CH_2Cl_2 cast) 3067, 3025, 2908, 2862, 1453 cm^{-1}; ^1H NMR (CD_2Cl_2, 400 MHz) δ 7.42-7.14 (m, 20 H, ArH), 4.92-4.77 (m, 4 H, 4 x PhCHHO), 4.67 (d, 1 H, J = 11.3 Hz, PhCHHO), 4.54-4.46 (m, 3 H, 3 x PhCHHO), 4.22 (m, 1 H, H-2), 3.76-3.48 (m, 6 H, H-3, H-4, H-5, H-6, H-7a, H-7b), 2.12 (dd, 1 H, J = 11.3, 10.0 Hz, H-1a), 1.93 (dd, 1 H, J = 11.3, 7.0 Hz, H-1b); ^{13}C NMR (CD_2Cl_2, 75 MHz) δ 139.1, 138.8, 138.6 and 138.0 (4 x ArC), 128.9, 128.7, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9 (20 x ArCH), 82.0, 79.2, 78.7, 75.6, 73.6 (5 x CH-sugar), 75.5, 75.2, 74.5, 73.7, 69.8 (5 x CH_2O), 26.5 (CH_2HgCl); MS (CI, NH_3) *m/z* (relative intensity) 556 (13%), 448 (8), 431; Anal. Calcd for $C_{35}H_{37}ClHgO_5$: C, 54.25; H, 4.82. Found: C, 54.17 H, 4.71.



(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)methyl iodide (84). A solution of **83** (4.70 g, 6.07 mmol) in dry CH_2Cl_2 (150mL) was stirred under an argon atmosphere for 0.5 h to remove traces of oxygen. Iodine (4.90 g, 19.3 mmol) was then added. After 6 h of stirring, a further portion of iodine (1.27 g, 5.00 mmol) was added and the stirring was continued for an additional 4 h. The reaction mixture was then treated with a 10 % aqueous sodium sulfite solution (140 mL) and stirred at room temperature for 1 h. The organic layer was separated and washed successively with 5 % aqueous KI (100 mL) and brine (100 mL) dried (Na_2SO_4) and concentrated *in vacuo.* The syrup obtained was subjected to flash chromatography (silica, petroleum ether-ethyl acetate, gradient, 20:1 to 10:1) to yield the title compound **84**[236] (3.4 g, 84 %) as a crystalline solid: mp 81-82 °C; IR ($CHCl_3$ cast) 3021, 2910, 2862, 1492, 1454 cm^{-1}; ^1H NMR (CD_2Cl_2, 400 MHz) δ 7.41-7.17 (m, 20 H, ArH), 4.86 (d, 1 H, J = 11.0 Hz, PhCHHO), 4.83 (d, 1 H, J = 11.0 Hz, PhCHHO), 4.77 (d, 1 H, J = 11.0 Hz, PhCHHO), 4.70 (d, 1 H, J = 11.0 Hz, PhCHHO), 4.65 (d, 1 H, *J* = 11.0 Hz, PhCHHO), 4.57 (d, 1 H, *J* = 11.0 Hz, PhCHHO), 4.55 (d, 1 H, *J* = 11.0 Hz, PhCHHO), 4.51 (d, 1 H, *J* = 11.0 Hz, PhCHHO), 4.18 (ddd, 1 H, *J* = 11.0, 5.0, 4.5 Hz, H-2), 3.76-3.64 (m, 4 H, H-4, H-5, H-7a, H-7b), 3.67-3.54 (m, 2 H, H-1a, H-3), 3.51 (ddd, 1 H, *J* = 10.0, 4.0, 2.5 Hz, H-6), 3.42 (dd, 1 H, *J* = 11.0, 11.0 Hz, H-1b); ^{13}C NMR (CD_2Cl_2, 75 MHz) δ 139.2, 138.9, 138.8 and 138.4 (4 x ArC), 128.8, 128.7, 128.2, 128.1, 128.0 and 127.9 (20 x ArCH), 81.6, 79.9, 78.1, 74.9, 72.0 (5 x CH-sugar), 75.4, 75.1, 73.8, 73.7, 69.6 (6 x CH_2); MS (CI, NH_3) *m/z* (relative intensity) 682 (MNH_4^+, 59%), 181 (17), 91 (100); Anal. Calcd for $C_{35}H_{37}IO_5$: C, 63.26; H, 5.61. Found: C, 63.08; H, 5.51.

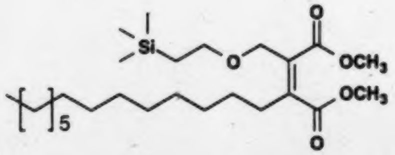


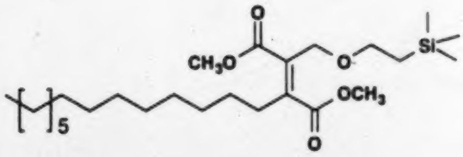


Dimethyl (*Z*)-2-methoxymethyl-3-tetradecylbutenedioate (85a) and dimethyl (*E*)-2-mthoxymethyl-3-tetradecylbutenedioate (85b). The general procedure for **40** was employed. CuBr•Me_2S (1.64 g, 8.00 mmol), tetradecylmagnesium bromide (8.00 mL of a 1.0 M solution in THF, 8.00 mmol) and freshly distilled DMAD (0.995 g, 7.00 mmol) were reacted as before. Freshly distilled methoxymethyl chloride (MOMCl) (1.69 g, 21.0 mmol) was added at - 78 °C and the reaction was warmed to 0 °C over 3 h and stirred at this temperature overnight. The usual work up gave 3.0 g of a yellow residue which was subjected to flash column chromatography (SiO_2, petrolum ether-ether, 8.5:1.5) to give **85a** (510 mg, 19 %) and **85b** (1.46 g, 54 %) both as colorless oils. Compound **40a** (220 mg, 8 %) was also produced. For **85a**: IR ($CHCl_3$, cast) 2927, 2865, 1729, 1435 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.19 (s, 2 H, $CH_3OCH_2C{=}C$), 3.76 and 3.74 (2s, 6 H, 2 x CO_2CH_3), 3.33 (s, 3 H, CH_3O), 2.39 (t, 2 H, *J* = 7.3 Hz, $-CH_2CH_2C{=}C$), 1.45-1.15 (m, 24 H, $-(CH_2)_{12}-$), 0.85 (t, 3 H, *J* = 6.1 Hz, CH_3-chain); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 168.9 and 167.6 (2 x C=O), 143.6 and 132.5 (C=C), 67.7 ($CH_3OCH_2C{=}C$), 58.4

($\underline{C}H_3OCH_2$-), 52.2 and 52.1 (2 x $CO_2\underline{C}H_3$), 31.8, 30.2, 29.6, 29.5, 29.4, 29.3, 29.2, 28.1 and 22.6 (-($\underline{C}H_2)_{13}$-), 14.0 ($\underline{C}H_3$-chain); HRMS (EI) Calcd for $C_{22}H_{40}O_5$ 384.2876, found 384.2865

For **85b**: IR ($CHCl_3$ cast) 2925, 2854, 1732, 1458, 1434 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 4.24 (s, 2 H, $CH_3OC\underline{H}_2C{=}C$), 3.77 and 3.76 (2s, 6 H, 2 x $CO_2C\underline{H}_3$), 3.27 (s, 3 H, $C\underline{H}_3O$-), 2.41 (t, 2 H, J = 7.5 Hz, -$CH_2C\underline{H}_2C{=}C$), 1.40 (qn, 2 H, -$C\underline{H}_2CH_2C{=}C$), 1.22 (m, 22 H, -($C\underline{H}_2)_{11}$-), 0.85 (t, 3 H, J = 6.6 Hz, $C\underline{H}_3$-chain); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 168.5 and 167.6 (2 x $\underline{C}{=}O$), 139.9 and 134.7 ($\underline{C}{=}\underline{C}$), 70.0 ($CH_3O\underline{C}H_2C{=}C$), 58.2 ($\underline{C}H_3OCH_2$-), 51.8 and 51.7 (2 x $CO_2\underline{C}H_3$), 31.8, 31.4, 29.6, 29.5, 29.4, 29.3, 29.2, 28.3 and 22.6 (-($CH_2)_{13}$-), 13.9 ($\underline{C}H_3$-chain); HRMS (EI) Calcd for $C_{22}H_{40}O_5$ 384.2876, found 384.2872. Anal. Calcd for $C_{22}H_{40}O_5$: C, 68.71; H, 10.48. Found: C, 68.90; H, 10.25.

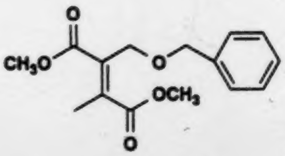




Dimethyl (*Z*)-2-(2'-Trimethylsilylethoxy)methyl-3-tetradecylbutenedioate (86a) and dimethyl (*E*)-2-(2'-trimethylsilylethoxy)methyl-3-tetradecylbutenedioate (86b). The procedure for compound **40** was followed. Thus, $CuBr{\bullet}Me_2S$ (4.11 g, 20.0 mmol), tetradecylmagnesium bromide (20.0 mL of a 1.0 M solution in THF, 20.0 mmol) and freshly distilled DMAD (2.56 g, 18.0 mmol) were reacted as before. Freshly distilled trimethylsilylethoxymethyl chloride (6.67 g, 40.0 mmol) was added at - 78 °C, the reaction was warmed to -40 °C and stirred for 3 h and then at 0 °C for 5 h. The usual work up gave a yellow residue. Flash column chromatography (SiO_2, petrolum ether-ether, 85:15) gave **86a** (2.33 g, 28 %) and **86b** (4.59 g, 54%). For **86a** (colorless oil, R_f = 0.42): IR ($CHCl_3$ cast) 2950, 2923, 1730 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.21 (s, 2 H, $OC\underline{H}_2C{=}C$), 3.75 and 3.74 (2s, 6 H, 2 x $OC\underline{H}_3$), 3.52 (dd, 2 H, J = 9.2, 8.1 Hz,

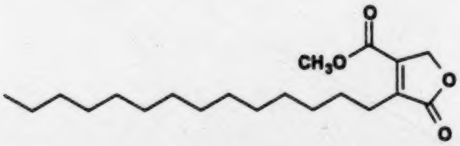
$(CH_3)_3Si$-CH_2-$C\underline{H}_2O$-), 2.39 (t, 2 H, J = 7.3 Hz, -$CH_2C\underline{H}_2C{=}C$), 1.47-1.20 (m, 24 H, -$(C\underline{H}_2)_{12}$-), 0.94-0.85 (m, 5 H, $(CH_3)_3Si$-$C\underline{H}_2$-CH_2 and $C\underline{H}_3$-chain), -0.01 (s, 9 H, $(C\underline{H}_3)_3Si$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.0 and 167.9 (2 x $\underline{C}{=}O$), 142.6 and 133.5 ($\underline{C}{=}\underline{C}$), 68.2 and 65.7 ($\underline{C}H_2O\underline{C}H_2C{=}C$), 52.1 and 51.7 (2 x $O\underline{C}H_3$), 31.9, 30.2, 29.7, 29.5, 29.4, 29.3, 28.3, 22.7 and 18.1 (-$(\underline{C}H_2)_{13}$ and $(CH_3)_3Si$-$\underline{C}H_2$-), 14.1 ($\underline{C}H_3$-chain) , -1.5 ($(\underline{C}H_3)_3Si$-); HRMS (EI) Calcd for $C_{26}H_{50}O_5Si$ 470.3427, found 470.3432. Anal. Calcd for $C_{26}H_{50}O_5Si$: C, 66.34; H, 10.71. Found: C, 66.63; H, 10.80.

For **86b** (colorless oil, R_f = 0.60.): IR ($CHCl_3$ cast) 2952, 2925, 2854, 1732, 1433 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 4.28 (s, 2 H, $OC\underline{H}_2C{=}C$), 3.77 and 3.76 (2s, 6 H, 2 x $OC\underline{H}_3$), 3.47 (dd, 2 H, J = 8.7, 8.1 Hz, $(CH_3)_3Si$-CH_2-$C\underline{H}_2O$-), 2.39 (t, 2 H, J = 7.5 Hz, -$CH_2C\underline{H}_2C{=}C$), 1.40 (qn, 2 H, -$C\underline{H}_2CH_2C{=}C$), 1.29-1.20 (m, 22 H, -$(C\underline{H}_2)_{11}$-), 0.90-0.85 (m, 5 H $(CH_3)_3Si$-$C\underline{H}_2$-CH_2- + $C\underline{H}_3$-chain), -0.02 (s, 9 H, $(C\underline{H}_3)_3Si$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 168.5 and 167.6 (2 x $\underline{C}{=}O$), 138.9 and 135.5 ($\underline{C}{=}\underline{C}$), 67.8 ($\underline{C}H_2O\underline{C}H_2C{=}C$), 51.6 and 51.5 (2 x $O\underline{C}H_3$), 31.8, 31.3, 29.5, 29.3, 29.2, 29.1, 28.3, 22.5 and 17.8, (-$(\underline{C}H_2)_{13}$ and $(CH_3)_3Si$-$\underline{C}H_2$-), 13.9 ($\underline{C}H_3$-chain), -1.6 ($(\underline{C}H_3)_3Si$-); HRMS (EI) Calcd for $C_{26}H_{50}O_5Si$ 470.3427, found 470.3422. Anal. Calcd for $C_{26}H_{50}O_5Si$: C, 66.34; H, 10.71. Found: C, 66.65; H, 10.95.

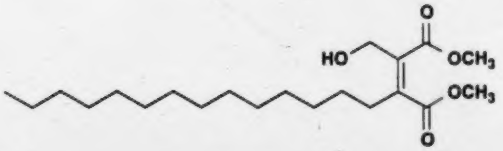


Dimethyl (*E*)-2-benzyloxymethyl-3-methylbutenedioate (87b). The general procedure for preparing **40** was followed. Thus, $CuBr{\bullet}Me_2S$ (1.64 g, 8.00 mmol), methylmagnesium bromide (2.67 mL of a 3.0 M solution in THF, 8.00 mmol) and freshly distilled DMAD (0.995 g, 7.00 mmol) were reacted as before. Benzyloxymethyl chloride (2.19 g, 14.0 mmol) was added at - 78 °C and the reaction was warmed to 10 °C overnight. Quenching and working up the reaction in the usual way gave a yellow oil.

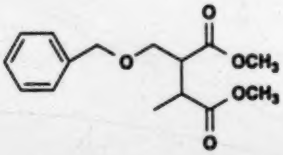
Purification by flash column chromatography (SiO_2, petrolum ether-ether, 8:2) gave **87b** (625 mg, 32 %) as a colorless oil: IR ($CHCl_3$ cast) 2952, 2860, 1727, 1453, 1434 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.35-7.22 (m, 5 H, $C_6\underline{H}_5$-), 4.49 (s, 2 H, $C\underline{H}_2O$), 4.44 (d, 2 H, J = 1.3 Hz, $C\underline{H}_2O$), 3.78 and 3.69 (2s, 6 H, 2 x $OC\underline{H}_3$), 2.06 (t, 3 H, J = 1.2 Hz, $C\underline{H}_3C{=}C$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 168.4 and 167.6 (2 x $\underline{C}{=}O$), 137.7, 136.4 and 134.4 ($\underline{C}{=}\underline{C}$ + $Ar\underline{C}$), 128.1, 127.5 and 127.4 (5 x $Ar\underline{C}H$), 72.4 and 67.4 (2 x $\underline{C}H_2O$), 51.8 and 51.7 (2 x $OC\underline{H}_3$), 17.3 ($\underline{C}H_3C{=}C$); HRMS (EI) Calcd for $C_{14}H_{15}O_4$ (M^+ - CH_3O) 247.0970, found 247.0967. Anal. Calcd for $C_{15}H_{18}O_5$: C, 64.74; H,6.52. Found: C, 64.65; H, 6.49.



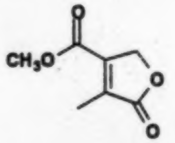
4-Carbomethoxy-3-tetradecyl-2-(5*H*)-furanone (88). To a solution of **86b** (26.1 mg, 0.055 mmol) in acetonitrile (1 mL) was added $BF_3{\bullet}Et_2O$ (5.5 µL, 0.045 mmol) and the mixture was stirred at room temperature for 1 h. The reaction was stopped by the addition of ice-cold H_2O (3 mL). After extracting with CH_2Cl_2 (5 x 5 mL) drying, and evaporation of solvent *in vacuo*, pure **88** (18 g, 97 %) was obtained as white solid: m.p 42-43 °C; IR ($CHCl_3$ cast) 2917, 2849, 1763, 1717, 1439 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.88 (t, 2 H, J = 1.4 Hz, -$C\underline{H}_2O$-), 3.84 (s, 3 H, $OC\underline{H}_3$), 2.67 (t, 2 H, J = 9.2 Hz, -$CH_2C\underline{H}_2C{=}C$), 1.53 (qn, 2 H, -$C\underline{H}_2CH_2C{=}C$), 1.35-1.21 (m, 22 H, -$(C\underline{H}_2)_{11}$), 0.87 (t, 3 H, J = 6.5 Hz, $C\underline{H}_3$-chain); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.3 ($\underline{C}{=}O$, lactone), 162.2 ($\underline{C}{=}O$, ester), 144.0 and 142.0 ($\underline{C}{=}\underline{C}$), 69.5 ($\underline{C}H_2O$), 52.4 ($O\underline{C}H_3$), 32.0, 29.7, 29.6, 29.5, 29.4, 29.3, 28.1, 24.9 and 22.7 (-$(\underline{C}H_2)_{13}$), 14.1 ($\underline{C}H_3$-chain); HRMS (EI) Calcd for $C_{20}H_{34}O_4$ 338.2457, found 338.2454, and for $C_{18}H_{31}O_2$ (M^+ - $C_2H_3O_2$, 100 %) 279.2324, found 279.2327. Anal. Calcd for $C_{20}H_{34}O_4$: C, 70.97; H, 10.12. Found: C, 70.65; H, 10.24.



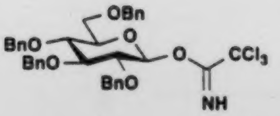
Dimethyl (*Z*)-2-hydroxymethyl-3-tetradecylbutenedioate (89). To a solution of **86a** (2.10 g, 4.46 mmol) in dry CH_3CN (40 mL) was added distilled $BF_3•Et_2O$ (0.493 mL, 4.01 mmol) at room temperature and the mixture was stirred for 2 h. The reaction was then quenched by adding ice-cold H_2O (30 mL) and further stirred for 5 min. Extraction with CH_2Cl_2 (4 x 50 mL), drying (Na_2SO_4) and evaporation of solvent *in vacuo* gave 1.69 g of crude product. Further purification by flash column chromatography (SiO_2, petrolum ether-ether, 6:4) gave **89** (1.56 g, 95 %) as a white solid: mp 47-48 °C; IR ($CHCl_3$ cast) 3442, 2924, 2853, 1724, 1462, 1434 cm^{-1}; ^1H NMR (CD_2Cl_2, 360 MHz) δ 4.35 (d, 2 H, J = 6.5 Hz, $C\underline{H}_2OH$), 3.74 (s, 6 H, 2 x $OC\underline{H}_3$), 2.39 (t, 2 H, J = 7.5 Hz, $-CH_2C\underline{H}_2C{=}C$), 2.34 (t, 1 H, J = 6.5 Hz, OH), 1.43 (qn, 2 H, $-C\underline{H}_2CH_2C{=}C$), 1.34-1.20 (br s, 22 H, $-(C\underline{H}_2)_{11}$), 0.88 (t, 3 H, J = 6.5 Hz, $C\underline{H}_3$-chain); ^{13}C NMR (CD_2Cl_2, 100 MHz) δ 169.7 and 168.0 (2 x $\underline{C}{=}O$), 143.9 and 133.6 ($\underline{C}{=}\underline{C}$), 58.8 ($\underline{C}H_2OH$), 52.5 and 52.4 (2 x $O\underline{C}H_3$), 32.3, 30.7, 30.1, 30.0, 29.9, 29.8, 29.7, 28.7 and 23.1 ($-(\underline{C}H_2)_{13}$), 14.3 ($\underline{C}H_3$-chain); HRMS (EI) Calcd for $C_{21}H_{38}O_5$ 370.2719, found 370.2719 and for $C_{20}H_{35}O_4$ (M^+ - CH_3O, 100 %) 339.2535, found 339.2529. Anal. Calcd for $C_{21}H_{38}O_5$: C, 68.07; H, 10.34. Found: C, 68.11; H, 10.42.



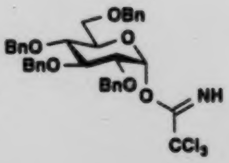
(2*S*, 3*S*) and (2*R*, 3*R*) Dimethyl 2-benzyloxymethyl-3-methylbutanedioate (90). A mixture of **87b** (72.0 mg, 0.259 mmol) and 10 % palladium on carbon (20 mg) in MeOH (5 mL) was stirred under a hydrogen atmosphere for 15 min. Even though tlc indicated that the reaction was not complete, the reaction mixture was filtered through a small pad of Celite, the solid was washed with MeOH and the filtrate was concentrated *in vacuo* to yield a clear oil. Flash chromatography (SiO_2, petrolum ether-ether, 8:2) yielded compound **90** (30.7 mg, 42 %) as a colorless oil: IR ($CHCl_3$ cast) 2951, 2864, 1737 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.39-7.21 (m, 5 H, $C_6\underline{H}_5$), 4.49 (d, 2 H, J = 2.5 Hz, $C\underline{H}_2O$), 3.72 (m, 2 H, $C\underline{H}_2O$), 3.69 and 3.65 (2s, 6 H, 2 x $OC\underline{H}_3$), 3.06-2.95 (m, 2 H, CO-C$\underline{H}$-C$\underline{H}$-CO), 1.16 (d, 3 H, J = 6.9 Hz, $C\underline{H}_3CH$-); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.7 and 173.2 (2 x $\underline{C}$=O), 137.9 (Ar$\underline{C}$), 128.4, 127.7 and 127.6 (5 x Ar$\underline{C}$H), 73.2 and 67.6 (2 x $\underline{C}H_2O$), 51.9 and 51.8 (2 x $O\underline{C}H_3$), 47.9 and 38.1 (-$\underline{C}$H-$\underline{C}$H), 14.3 ($\underline{C}H_3CH$); HRMS (EI) Calcd for $C_{15}H_{20}O_5$ 280.1311, found 280.1312. Anal. Calcd for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19. Found: C, 64.32; H, 7.47.



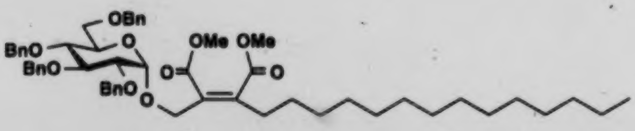
4-Carbomethoxy-3-methyl-2-(5*H*)-furanone (91). To a solution of diester **87b** (58.7 mg, 0.211 mmol) in dry CH_2Cl_2 (1 mL) was added trimethylsilyl iodide (39 µL, 0.274 mmol) at room temperature. After 2 h, the reaction mixture was worked up by the addition of methanol (2 mL), followed by concentration *in vacuo*. The residue was taken up in ether (10 mL) and the ether solution was washed with aqueous sodium bisulfite (5 mL), $NaHCO_3$ (5 mL) and brine (5 mL). Drying (Na_2SO_4) and concentration of the organic layer gave a yellow residue which was purified by column chromatography (SiO_2, petrolum ether-ether, 7:3) to give lactone **91** (15 mg, 46 %) along with some unreacted starting material. For **91**: IR ($CHCl_3$ cast) 2955, 2861, 1755 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.90 (q, 2 H, J = 2.4 Hz,- $C\underline{H}_2O$), 3.88 (s, 3 H, $OC\underline{H}_3$) , 2.23 (t, 3 H, $C\underline{H}_3C$=C); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.6 ($\underline{C}$=O, lactone), 162.3 ($\underline{C}$=O, ester), 144.3, and 137.6 ($\underline{C}$=$\underline{C}$), 69.6 (-$\underline{C}H_2O$), 42.4 ($O\underline{C}H_3$), 10.7 ($\underline{C}H_3C$=C); HRMS (EI) Calcd for $C_7H_8O_4$ 156.0423, found 156.0427.



O-(2,3,4,6-Tetra-O-benzyl-β-D-glucopyranosyl)trichloroacetimidate (92a). The literature[244] procedure was modified. Thus, to a solution of 2,3,4,6-tetra-*O*-benzyl-D-glucose (100 mg, 0.185 mmol) in dry CH_2Cl_2 (2 mL) was added K_2CO_3 (94.4 mg, 0.683 mmol) and trichloroacetonitrile (534 mg, 3.71 mmol). The suspension was vigorously stirred for 3.5 h at room temperature under an argon atmosphere. The mixture was filtered through celite, washed with CH_2Cl_2 (10 mL) and the filtrate was concentrated *in vacuo* to give an oil. Purification by flash column chromatography (SiO_2, petrolum ether-ether, gradient, 3:1 to 1:3) afforded the β-anomer **92a** (100 mg, 79 %) as a semi-solid and the α-anomer **92b** (21 mg, 16 %) as an oil (see compound **92b** below). For **92a** $[\alpha]_D$ +3.1° (*c* 0.4, $CHCl_3$); IR ($CHCl_3$ cast) 3337, 3030, 1670 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.82 (s, 1 H, N$\underline{H}$), 7.46-7.24 (m, 20 H, 4 x $C_6\underline{H}_5$), 5.93 (d, 1 H, *J* = 7.0 Hz, $\underline{H}$-1), 5.09-4.63 (m, 8 H), 3.88-3.74 (m, 6 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.0 ($\underline{C}$=NH), 138.3, 138.0, 137.9 and 137.8 (4 x Ar $\underline{C}$), 128.2, 127.8, 127.7, 127.6, 127.5 and 127.4 (Ar$\underline{C}$H), 98.2 ($\underline{C}$-1), 90.92 ($\underline{C}Cl_3$), 84.4, 80.8, 77.1 and 75.7 (4 x $\underline{C}$H), 75.4, 74.8, 74.7, 73.2 and 68.1 (5 x $\underline{C}H_2O$), MS (ES) *m/z* (relative intensity) 708.1 (MNa^+, 100%); Anal. Calcd for $C_{36}H_{36}Cl_3NO_6$: C, 63.12; H, 5.30; N, 2.04. Found: C, 63.25; H, 5.09; N, 2.02.

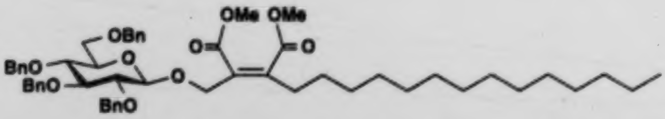


O-(2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosyl)trichloroacetimidate (92b). The literature[245] procedure was modified. Thus, to a solution of 2,3,4,6-tetra-*O*-benzyl-D-glucose **80** (3.00 g, 5.55 mmol) in dry CH_2Cl_2 (25 mL) was added trichloroacetonitrile (3.61 g, 25.0 mmol) and sodium hydride (222 mg, 60 % dispersion in oil, 5.55 mmol, washed with 3 x 3 mL of dry petrolum ether before use) and the mixture was stirred at room temperature. After 30 min a second portion of sodium hydride (222 mg, 5.55 mmol) was added and the mixture was stirred for 1h. The reaction mixture was then filtered through celite and the filtrate was concentrated *in vacuo* to give an orange syrup. Purification by flash column chromatography (SiO_2, petrolum ether-ether, 3:2) afforded **92b** (3.1 g, 82 %) as a colorless foam: IR ($CHCl_3$, cast) 3332, 3029, 1668 cm^{-1}; ^1H NMR (CD_2Cl_2, 360 MHz) δ 8.68 (s, 1 H, N$\underline{H}$), 7.41-7.20 (m, 20 H, 4 x $C_6\underline{H}_5$), 6.53 (d, 1 H, *J* = 3.4 Hz, $\underline{H}$-1), 4.98-4.48 (m, 8 H), 4.06-3.77 (m, 6 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.3 ($\underline{C}$=NH), 138.6, 138.0, 137.9 and 137.8 (4 x Ar$\underline{C}$), 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5 and 127.4 (Ar$\underline{C}$H), 94.3 ($\underline{C}$-1), 91.1 ($\underline{C}Cl_3$), 91.3, 79.3, 76.8 and 73.1 (4 x $\underline{C}$H), 75.6, 75.3, 73.4, 72.8 and 68.0 (5 x $C\underline{H}_2O$); HRMS (ES) calcd for $C_{36}H_{36}Cl_3NO_6Na$ (MNa^+, 100%) 706.1506, found 706.1510. Anal. Calcd for $C_{36}H_{36}Cl_3NO_6$: C, 63.12; H, 5.30; N, 2.04. Found: C, 63.45; H, 5.42; N, 1.97.

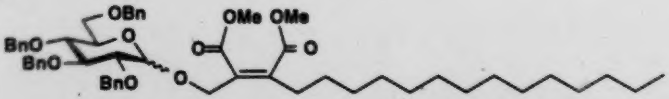


***O*-(Dimethyl (*Z*)-2-oxymethyl-3-tetradecylbutenedioate)-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (93a).** Anhydrous conditions are extremely important for the efficiency of this reaction. A mixture of imidate **92a** (224 mg, 0.327 mmol) and **89** (92.6 mg, 0.250 mmol) in dry ether (5 mL) was stirred in the presence of powdered activated molecular sieves (type 4Å) for 30 min. Trimethylsilyl triflate (21.8 mg, 0.0981 mmol) was then introduced to the reaction mixture at -20 °C. After stirring for 4 h at -20 °C, the reaction mixture was diluted with ether (10 mL) and filtred through celite. The filtrate was washed with a saturated solution of $NaHCO_3$ (10 mL) and brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give the crude product. Purification by column chromatography (SiO_2, petrolum ether-ether, 7:3) gave **93a** (173 mg, 77 %) as a colorless oil: $[\alpha]_D$ +4.25° (*c* 0.5, $CHCl_3$); IR ($CHCl_3$ cast) 2924, 2853, 1727, 1453 cm^{-1};

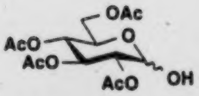
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.42-7.17 (m, 20 H, 4 x $C_6\underline{H}_5$), 5.01 (d, 1 H, J = 10.8 Hz), 4.93 (d, 1 H, J = 3.3 Hz, $\underline{H}$-1) , 4.85 (dd, 1 H, J = 13.7, 11.1 Hz, $\underline{H}$- 3), 4.71 (d, 1 H, J = 6.7 Hz), 4.64 (d, 1 H, J = 12.0 Hz), 4.51 (m, 3 H), 4.63 (d, 1 H, J = 11.9 Hz), 3.99 (t, 1 H, J = 9.1 Hz), 3.82 (s, 3 H, $C\underline{H}_3OCO$), 3.80-3.59 (m, 7 H), 3.72 (s, 3 H, $C\underline{H}_3OCO$), 2.52 (m, 2 H, $C{=}CC\underline{H}_2$- chain), 1.48-1.21 (m, 24 H, -$(C\underline{H}_2)_{12}$), 0.93 (t, 3 H, J = 6.3 Hz, $C\underline{H}_3$-chain); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 169.3 and 166.9 (2 x $\underline{C}{=}O$), 146.2, 138.8, 138.3, 138.2, 137.9 and 130.0 ($\underline{C}{=}\underline{C}$ + 4 x Ar$\underline{C}$)), 128.2, 127.9, 127.7, 127.6, 127.5, 127.4 and 127.3 (Ar$\underline{C}$H), 96.7 ($\underline{C}$-1), 81.8, 79.8, 77.5 and 70.6 (4 x $\underline{C}$H), 75.6, 74.9, 73.4, 72.6, 68.3 and 62.8 (6 x $\underline{C}H_2O$), 52.1 and 52.0, (2 x $O\underline{C}H_3$), 31.8, 30.8, 29.6, 29.4, 29.3, 29.2, 28.1 and 22.6 (-$(\underline{C}H_2)_{13}$-), 14.0 ($\underline{C}H_3$-chain); MS (ES) *m/z* (relative intensity) 915.4 (MNa^+, 100%). Anal. Calcd for $C_{55}H_{72}O_{10}$: C, 73.96; H, 8.13. Found: C, 73.87; H, 8.00.

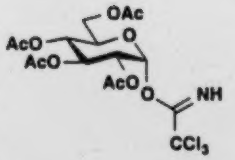


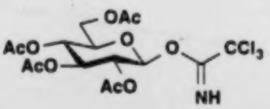
O-(Dimethyl (*Z*)-2-oxymethyl-3-tetradecylbutenedioate)-2,3,4,6-tetra-O-benzyl-β-D-glucopyranoside (93b). A mixture of alcohol **89** (43.6 mg, 0.118 mmol) and α-imidate **92b** (161 mg, 0.236 mmol) in CH_2Cl_2 (4 mL) was stirred in the presence of powdered activated molecular sieves (type 4 Å) for 30 min. The mixture was cooled to -20 °C, $BF_3{\bullet}Et_2O$ (28.9 μL, 0.236 mmol) was added and stirring was continued for 2 h. The reaction mixture was then warmed to room temperature and stirred for 10 min before it was diluted with CH_2Cl_2 (10 mL) and filtered through celite. The filtrate was washed with a saturated solution of $NaHCO_3$ (10 mL) and brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give the crude product. Purification by flash column chromatography (SiO_2, petrolum ether-ether, 7:3) afforded the title compound **93b** (93 mg, 88 %) as a colorless oil: $[\alpha]_D$ +0.38° (c 0.9, $CHCl_3$); IR ($CHCl_3$ cast) 2924, 1727 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.42-7.18 (m, 20 H, 4 $C_6\underline{H}_5$), 4.97-4.48 (m, 11 H), 3.81 (s, 3 H, C$\underline{H}_3$OCO), 3.80-3.62 (m, 4 H), 3.78 and 3.65 (2s, 6 H, 2 x C$\underline{H}_3$OCO), 3.59-3.42 (m, 2 H), 2.56-2.45 (m, 2 H, allylic C$\underline{H}_2$-chain), 1.45-1.22 (m, 24 H), 0.96 (t, 3 H, *J* = 6.2 Hz, CH_3-chain); ^{13}C NMR (CD_2Cl_2, 100 MHz) δ 169.6 and 167.5 (2 x $\underline{C}$=O), 146.2, 139.3, 139.1, 138.9, 138.8 and 130.9 ($\underline{C}$=$\underline{C}$ and 4 Ar$\underline{C}$), 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0 and 127.8 (Ar$\underline{C}$H), 102.9 (C-1), 84.9, 82.3, 78.1, 75.8, 75.3, 75.2, 74.7, 73.8, 69.4 and 64.3 (4 x $\underline{C}$H and 6 x $\underline{C}H_2$O), 52.5 (2 x O$\underline{C}H_3$), 32.3, 30.9, 30.1, 29.9, 29.8, 28.6 and 23.1 (-($\underline{C}H_2)_{13}$-), 14.3 ($\underline{C}H_3$-chain); MS (FAB, Cleland) *m/z* (relative intensity) 894 (MH^+, 6%); HRMS (ES) calcd for $C_{55}H_{72}O_{10}Na$ (MNa^+, 100%) 915.5023, found 915.5015.



93a and 93b *via* 1-*O*-alkylation of tetra-*O*-benzylglucose. To a solution of 2,3,4,6-tetra-*O*-benzyl-D-glucose (20.0 mg, 0.0370 mmol) in THF (1 mL) was added NaH (1.63 mg, 60 % dispersion in oil, 0.041 mmol, washed with 2 x 0.5 mL of dry petroleum ether before use) followed by mesylate **112** (16.6 mg, 0.0370 mmol). The mixture was stirred at room temperature under argon for 2 h then worked-up by pouring it into H_2O (3 mL). Ether was added and the layers were separated. The aqueous layer was extracted with ether (2 x 5 mL) and the combined organic extracts were washed with brine (8 mL) and dried (Na_2SO_4). Concentration *in vacuo* gave 29 mg of residue. Purification by flash column chromatography (SiO_2, petrolum ether-ether, 8:2) gave 6 mg (18 %) of the alkylation product as a mixture of α and β anomers. No attempts were made towards the separation of the two isomers, **93a** and **93b**.





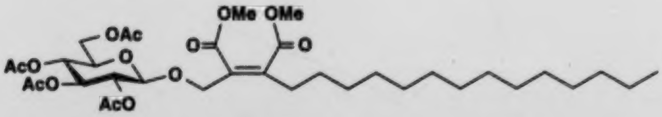


2,3,4,6-Tetra-*O*-acetyl-D-glucose (96), *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)trichloroacetimidate (97a), and *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl) trichloroacetimidate (97b). To a solution of 1,2,3,4,6-penta-*O*-acetyl-β-D-glucose (1.00 g, 2.48 mmol) in dry DMF (5 mL) was added hydrazine acetate (260 mg, 2.82 mmol) at room temperature and the mixture was stirred for 30 min. After dilution with ethyl acetate (20 mL), the mixture was washed with H_2O (2 x 15 mL), a saturated solution of $NaHCO_3$ (15 mL) and brine (15 mL). Drying (Na_2SO_4) and concentration of the organic layer gave **96** (832 mg, 96 %) as a white waxy foam. The ^{1}H NMR spectrum showed that the product was fairly pure (mainly α anomer) and hence it was used in the next step without further purification: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.40 (t, 1 H, *J* = 9.8 Hz), 5.30 (d, 1 H, *J* = 3.6, H-1), 4.94 (m, 1 H), 4.73 (dd, 1 H, *J* = 10.1, 3.5 Hz), 4.17-4.09 (m, 2 H), 4.03-3.94 (m, 2 H), 1.96, 1.95, 1.90 and 1.89 (4 s, 12 H, 4 C$\underline{H}_3$CO).

To a solution of **96** (583 mg, 1.67 mmol) and trichloroacetonitrile (725 mg, 5.02 mmol) in CH_2Cl_2 (5 mL) was added freshly ground and heat-dried K_2CO_3 (389 mg, 2.82 mmol). The suspension was stirred at room temperature for 1.15 h and then diluted with CH_2Cl_2 (10 mL) and filtered. The filtrate was concentrated *in vacuo* to give a yellow foam. The β-anomer **97b** (161 mg, 20 %) crystallized out from petroleum ether-ethyl acetate (3:1) when attempting to chromatograph the crude product. The remaining oil was chromatographed (SiO_2, petrolum ether-ethyl acetate, 2:1) to give the α-anomer **97a** (493 mg, 54 %) as a white foam and 10 % of starting material **96**.

For the α-anomer **97a**^{246}: IR ($CHCl_3$ cast) 3318, 1755, 1678 cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 8.69 (s, 1 H, N$\underline{H}$), 6.43 (d, 1 H, *J* = 3.6 Hz, $\underline{H}$-1), 5.44 (t, 1 H, *J* = 9.9 Hz, $\underline{H}$-3), 5.09-4.99 (m, 2 H,), 4.19-3.96 (m, 3 H), 1.95, 1.93, 1.91, 1.89 (4 s, 12 H, 4 x $C\underline{H}_3CO$); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 170.7, 170.2, 169.4 and 169.0 (4 x $CH_3\underline{C}O$), 161.0 ($\underline{C}$=NH), 95.6 ($\underline{C}$-1), 90.4 ($\underline{C}Cl_3$), 72.7, 72.6, 70.2 and 67.9 (4 x $\underline{C}H$), 61.6 ($\underline{C}H_2O$), 20.7, 20.6 and 20.5 (4 x $\underline{C}H_3CO$); HRMS (EI) Calcd for $C_{14}H_{19}O_9$ $(M- C_2HCl_3NO)^+$ 331.1029, found 331.1036.

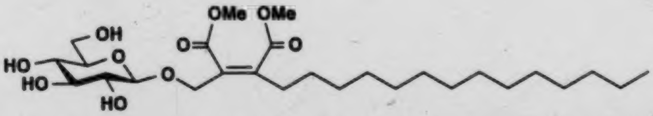
For the β-anomer **97b**: mp 155-156 °C, (lit.[247]. mp 154-155 °C); IR ($CHCl_3$ cast) 3319, 1752, 1677 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 8.78 (s, 1 H, $N\underline{H}$), 5.86 (distorted d, 1 H, $\underline{H}$-1), 5.29-5.15 (m, 3 H), 4.29 (dd, 1 H, J = 12.5, 4.4 Hz, $\underline{H}$-6a), 4.13 (dd, 1 H, J = 12.5, 2.4 Hz, $\underline{H}$-6b), 3.90-3.85 (m, 1 H, $\underline{H}$-5), 2.05, 2.01, 1.99 and 1.98 (4s, 12 H, 4 x $C\underline{H}_3CO$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.1, 169.6, 169.4 and 169.1 (4 x $CH_3\underline{C}O$), 160.3 ($\underline{C}$=NH), 92.6 ($\underline{C}$-1), 90.4 ($\underline{C}Cl_3$), 69.8, 69.6, 69.4 and 67.5 (4 x $\underline{C}H$), 61.1 ($\underline{C}H_2O$), 20.3, 20.2 and 20.1 (4 x $\underline{C}H_3CO$); HRMS (EI) Calcd for $C_{14}H_{19}O_{10}$ $(M- C_2HCl_3N)^+$ 347.0978, found 347.0970.



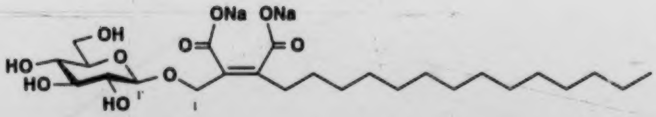
***O*-(Dimethyl (*Z*)-2-oxymethyl-3-tetradecylbutenedioate)-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (98).** The general glycosylation procedure given above was followed. Thus, a mixture of alcohol **89** (44.9 mg, 0.121 mmol) and α-imidate **97a** (71.7 mg, 0.146 mmol) in CH_2Cl_2 (4 mL) was stirred in the presence of powdered activated 4 Å molecular sieves (150 mg) for 30 min. The mixture was cooled to -20 °C, $BF_3 \bullet Et_2O$ (17.9 μL, 0.146 mmol) was added and stirring was continued for 1 h at the same temperature and 10 min at room temperature. The reaction was diluted with CH_2Cl_2 (5 mL) and filtered through celite. The filtrate was washed with a saturated solution of $NaHCO_3$ (6 mL) and H_2O (6 mL). Drying (Na_2SO_4) and concentration *in vacuo* afforded the crude product. Purification by flash column chromatography (SiO_2, petrolum ether-ethyl acetate, 3:1) gave glycoside **98** (51 mg, 60 %; 84 % yield based on consumed starting material) along with recovered alcohol **89** (17 mg, 31 %). Glycoside **98** was isolated as a colorless oil: $[\alpha]_D$ -4.1° (*c* 0.2, $CHCl_3$); IR ($CHCl_3$ cast) 2926, 2855, 1758, 1638, 1435 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.18 (t, 1 H, *J* = 9.4 Hz, H-3), 5.07 (t, 1 H, *J* = 9.5 Hz, H-2), 4.94 (dd, 1 H, *J* = 9.5, 8.0 Hz, H-4), 4.55 (m, 2 H, -OCH_2C=C), 4.41 (d, 1 H, *J* = 12.2 Hz, H-1), 4.24 (dd, 1 H, *J* = 12.3, 4.6 Hz, H-6a), 4.13 (dd, 1 H, *J* =12.3, 2.4 Hz, H-6b), 3.76 and 3.73 (2 s, 6 H, 2 x OCH_3), 3.69-3.65 (m, 1 H, H-5), 2.41-2.46 (m, 2 H, C=CCH_2-chain), 2.07, 2.00, 1.99 and 1.98 (4 s, 12 H, 4 x CH_3CO), 1.43-1.25 (m, 24 H, -$(CH_2)_{12}$), 0.86 (t, 1 H, *J* = 6.6 Hz, CH_3- chain); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.5, 170.2, 169.3, 169.2, 169.0 and 166.9 (6 x C=O), 145.9 and 130.1, (C=C), 99.7 (C-1), 72.8, 71.9, 71.0 and 68.3 (4 x CH), 64.0 and 61.9 (2 x CH_2O), 52.2 and 52.1 (2 x CH_3OCO), 31.9, 30.5, 29.6, 29.5, 29.4, 29.3, 29.2, 28.0 and 22.6 (-$(CH_2)_{13}$-chain), 20.7 and 20.5 (4 x CH_3CO), 14.1 (CH_3- chain); HRMS (EI) Calcd for $C_{34}H_{53}O_{13}$ (M- $OCH_3)^+$ 669.3486, found 669.3492. Anal. Calcd for $C_{35}H_{56}O_{14}$: C, 59.98; H, 8.05. Found: C, 59.74; H, 8.25.

***O*-(Dimethyl (*Z*)-2-oxymethyl-3-tetradecylbutenedioate)-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (98) using acetobromo-α-D-glucose as the glycosyl donor.** A mixture of silver trifluoromethanesulfonate (14.1 mg, 0.0548 mmol), S-collidine (6.71, 0.548 mmol) and powdered 4 Å molecular sieves (30 mg) in dry CH_2Cl_2 (0.5 mL) was stirred at -78 °C for 10 min. Alcohol **89** (12.3 mg, 0.0332 mmol) in CH_2Cl_2 (0.5 mL) was then added. After 5 min 2, 3, 4, 6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (20.5 mg, 0.498 mmol) in CH_2Cl_2 (0.5 mL) was added and the resulting mixture was warmed to -30 °C and was stirred at this temperature for 30 min. It was then allowed to warm to room temperature and was stirred for 26 h. The reaction mixture was diluted with CH_2Cl_2 (5 mL), filtred through celite and washed with 0.1 M HCl (5 mL)and brine (5 mL). The organic layer was dried (Na_2SO_4) and concentrated *in vacuo* to give a residue. Flash column chromatography (SiO_2, petrolum ether-ether, gradient, 60:40 to 10:90) on the residue gave glycoside **98** (3 mg, 13%) as a colorless oil.

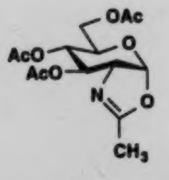
42 % of the alcohol starting material **89** was also recovered. The glycoside prepared by this method had identical spectral data to those given above.



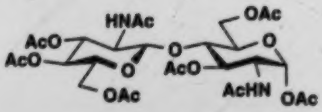
***O*-(Dimethyl (*Z*)-2-oxymethyl-3-tetradecylbutenedioate)-β-D-glucopyranoside (99).** To the protected derivative **98** (33.2 mg, 0.0471 mmol) in dry CH_3OH (2 mL) was added sodium methoxide (1.0 mg, 0.18 mmol) at 0 °C and the solution was stirred for 30 min at the same temperature and then at room temperature for 5 h. An excess amount of AG50W-X8 (H^+) ion exchange resin was added to quench the reaction. Filtration, followed by concentraion *in vacuo* afforded a residue which was purified by HPLC (Resolve C_{18} column, gradient, H_2O-CH_3CN, 100% H_2O to 100% CH_3CN over 50 min; R_t = 37.24 min) to give **98** (21.2 mg, 85 %) as an oil: $[\alpha]_D$ -2.28° (c = 1, $CHCl_3$); IR ($CHCl_3$ cast) 3387 (br), 2923, 2853, 1725, 1676, 1435 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.60 (d, 1 H, J = 12.7 Hz), 4.47 (d, 1 H, J = 12.1 Hz), 4.39-4.03 (br m, 4 H), 3.88-3.75 (br m, 2 H), 3.76 and 3.75 (2 s, 6 H, 2 x $C\underline{H}_3OCO$), 3.61-3.42 (br m, 2 H), 3.38-3.25 (br m, 2 H), 2.43 (br t, 2 H, J = 7.3 Hz, $C{=}CC\underline{H}_2$-chain), 1.42-1.20 (br m, 26 H, $-(C\underline{H}_2)_{13}$), 0.87 (t, 1 H, J = 6.4 Hz, $C\underline{H}_3$-chain); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 169.3 and 167.7 (2 x $\underline{C}{=}O$), 145.3 and 130.9 ($\underline{C}{=}\underline{C}$), 102.6 ($\underline{C}$-1), 76.3, 75.8, 73.5 and 70.1 (4 x $\underline{C}H$), 64.9 and 62.0 (2 x $\underline{C}H_2O$), 52.6 and 52.4 (2 x $\underline{C}H_3OCO$), 32.0, 30.5, 29.7, 29.6, 29.4, 28.2 and 22.7 ($-(\underline{C}H_2)_{13}$-chain), 14.2 ($\underline{C}H_3$- chain); MS (FAB, Cleland) *m/z* (relative intensity) 555.0 (MNa^+, 5%), 532.9 (MH^+, 1), 371.1 (15), 339.1 (100). Anal. Calcd for $C_{27}H_{48}O_{10}.H_2O$: C, 58.89; H, 9.15. Found: 58.72; H, 8.79.



(*Z*)-2-β-D-glucopyranosyloxymethyl-3-tetradecylbutenedioic acid disodium salt (100). To the di-ester derivative **99** (7.7 mg, 0.015 mmol) in $THF\text{-}H_2O$ (1:1, 0.5 mL)was added NaOH (35 μl of a 1.0 M solution, 0.035 mmol) and the mixture was stirred at room temperature for 2 days. The solvent was then removed and the residue was re-dissolved in H_2O and freeze-dried to give **100** (7.8 mg, 95%) as a white powder: IR (KBr) 3462, 3375, 1642,1590 cm^{-1}; ^{1}H NMR (CD_3OD + few drops of D_2O) δ 4.63 (d, 1 H, *J* = 12.0 Hz), 4.45-4.38 (m, 2 H, H-6'a and H-1a), 4.33 (d, 1 H, *J* = 7.8 Hz, H-1'), 3.85 (br d, 1 H, *J* = 11.5 Hz), 3.70-3.62 (m, 2H), 3.41-3.34 (m, 1 H, H-3'), 3.23-3.18 (m, 1 H, H-5'), 2.39-2.28 (m, 2 H, $-CH_2-CH_2C=C$), 1.45-1.20 (br m, 24 H, $-(CH_2)_{12}$), 0.88 (t, 3 H, *J* = 6.7 Hz, CH_3-chain); ^{13}C NMR (CD_3OD + few drops of D_2O, 400 MHz) δ 179.7 and 177.0 (2 x C=O), 148.2 and 130.0 (C=C), 102.0 (C-1'), 77.3, 77.2, 74.5 and 71.0 (4 x CHO), 66.6, 62.1 (CH_2OH and C-1'), 32.5, 31.6, 30.5, 30.3, 30.2, 30.1, 30.0, 29.9, 29.5 ($-CH_2-)_{13}$, 14.3 (CH_3-chain); HRMS (ES) Calcd for $C_{25}H_{42}O_{10}Na_2$ 548.2573, found 548.2578.

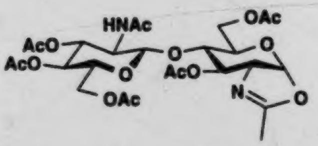


2-Methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)[2,1-*d*]-Δ^2oxazoline (102).[247] A mixture of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose (0.37 g, 0.94 mmol) and trimethylsilyl trifluoromethanesulfonate (0.27 mL, 1.41 mmol) in 1,2-dichloroethane (10 mL) was stirred at 50 °C under argon for 20 h. The reaction mixture was cooled to room temperature and triethylamine (5.0 mL) was added. The resulting mixture was concentrated *in vacuo* to give a brown syrup. Purification by flash chromatography (SiO_2, CH_2Cl_2-MeOH-Et_3N, 160:2:1) gave the oxazoline **102** (270 mg, 87 %) as a syrup: IR (CH_2Cl_2 cast) 1743, 1671, 1370 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.93 (d, 1 H, *J* = 7.4 Hz, $\underline{H}$-1), 5.20 (dd, 1 H, *J* = 2.4, 2.5 Hz, $\underline{H}$-3), 4.86 (ddd, 1 H, *J* = 9.0, 2.0, 1.0 Hz, $\underline{H}$-4), 4.16-3.99 (m, 3 H, $\underline{H}$-2, $\underline{H}$-6a, $\underline{H}$-6b), 3.58 (dt, 1 H, *J* = 9.0, 4.5 Hz, $\underline{H}$-5), 2.10-2.00 (m, 12 H, 3 x $C\underline{H}_3CO$ and $CH_3C{=}N$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.2, 169.2 and 169.1 (3 x $\underline{C}{=}O$), 166.4 ($\underline{C}{=}NH$), 99.3 ($\underline{C}$-1), 70.3, 69.2, 67.3, 64.8 and 63.3 ($C\underline{H}_2O$ and 4 x $\underline{C}H$), 20.8, 20.7 and 20.5 (3 x $\underline{C}H_3CO$), 13.8 ($\underline{C}H_3C{=}N$); MS (CI, NH_3) *m/z* (relative intensity) 330 (MH^+, 100%).



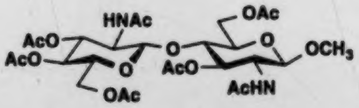
α-Chitobiose hexa-acetate (103)[249,251]. This experiment was done by Dr. Jane Taylor. Chitinase (300 units, 1500 mg @ 0.2 units / mg) was added to a vigorously stirred (mechanical stirrer) mixture of colloidal chitin (120 g) in a pH 6.3 buffer solution (570 mL, prepared by mixing appropriate volumes of 0.2M Na_2HPO_4 and 0.2M AcOH) and water (570 mL). The mixture was stirred at 40 °C for 15 days and then filtered. The solvent was evaporated from the filtrate and the resulting residue was dried overnight on a high vacuum pump. Acetic anhydride (150 mL) and anhydrous NaOAc were added and the mixture was stirred at 80 °C for 2 days. The solvent was evaporated and the residue was suspended in $CHCl_3$, washed with saturated aqueous $NaHCO_3$ solution, dried ($MgSO_4$) and the solvent was removed to give a crusty dark brown solid (approx. 90 g). This solid was divided into 2 portions and each half was separately purified by flash column chromatography (SiO_2, $CHCl_3$-MeOH, gradient, 40:1 to 10:1) to give 45 g of crude product. Recrystallization from MeOH gave pure chitobiose hexa-acetate (27.0 g). A second crop (6.3 g) was obtained by recrystallisation of the mother liquor.

Compounds eluted from the column after chitobiose hexa-acetate arose from incomplete acetylation (11.8 g) and can be retreated with acetic anhydride and NaOAc and purified as above to yield more chitobiose hexa-acetate **103**: (lit.[249] mp 305-306 (dec)); $[\alpha]_D$ +2.8° (*c* 0.4, $CHCl_3$); [lit.[249] $[\alpha]_D$ +55° (*c* 0.5, acetic acid)]; IR ($CDCl_3$ cast) 3287, 1746, 1666, 1538, 1434 m^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 6.08 (d, 1 H, *J* = 3.6 Hz, H-1), 6.03 (d, 1 H, *J* = 9.3 Hz, NH'), 5.71 (d, 1 H, *J* = 9.0 Hz, NH), 5.21 (dd, 1 H, *J* = 11.0, 9.0 Hz, H-3), 5.12 (t, 1 H, *J* = 9.5 Hz, H-3'), 5.04 (t, 1 H, *J* = 9.5 Hz, H-4'), 4.48 (d, 1 H, *J* = 8.4 Hz, H-1'), 4.44-4.31 (m, 3 H, H-2, H-6a, and H-6b), 4.18 (dd, 1 H, *J* = 12.2, 1.9 Hz, H-6b), 4.03-3.86 (m, 3 H, H-2', H-5', and H-6a), 3.74 (t, 1 H, *J* = 9.1 Hz, H-4), 3.62 (m, 1 H, H-5), 2.17, 2.13, 2.07, 2.04, 2.00, 1.99, 1.94 and 1.91 (8 s, 24 H, 8 x CH_3CO); HRMS (ES) Calcd for $C_{28}H_{40}N_2O_{17}Na$ (MNa^+) 699.2225, found 699.2214 and for $C_{28}H_{41}N_2O_{17}$ (MH^+) 677.23, found 677.2359. Anal. Calcd for $C_{28}H_{40}N_2O_{17}$: C, 49.70; H, 5.96; N, 4.14. Found: C, 49.68; H, 5.85; N, 4.13.



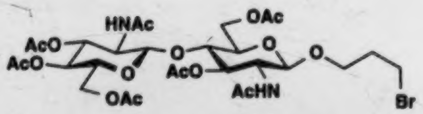
2-Methyl-[2-acetamido-4-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano]-[2,1-d]-2-oxazoline (104).[252] To a solution of **103** (500 mg, 0.739 mmol) in dry 1,2-dichloroethane (5 mL) was added trimethylsilyl trifluoromethanesulfonate (251 mg, 1.11 mmol) at room temperature under an argon atmosphere. The reaction mixture was stirred at 50 °C for 15 h, cooled and triethylamine (400 μL) was added. The mixture was concentrated *in vacuo* to about 2 mL and then applied to a column of silica gel and eluted with dichloromethane-methanol-triethylamine, 50:1.0:0.1 to give **104** (416 mg, 91 %) as an amorphous powder: [lit.[252] $[\alpha]_D$ -8° (*c* 1.0, $CHCl_3$)]; IR ($CHCl_3$ cast) 3280, 2933, 1743, 1672, 1370 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.96 (d, 1 H, *J* = 8.7 Hz, NH'), 5.88 (d, 1 H, *J* = 7.3, H-1), 5.63 (d, 1 H, *J* = 1.8 Hz, H-3), 5.19 (t, 1 H, *J* = 9.7 Hz, H-3'), 5.05 (t, 1 H, *J* = 9.7 Hz, H-4'), 4.73 (d, 1 H, J = 8.5 Hz, H-1'), 4.27 (dd, 1 H, *J* = 12.2, 4.7 Hz, H-

6b), 4.24 (dd, 1 H, *J* = 12.3, 4.4 Hz, H-6b'), 4.10 (m, 3 H, H-2, H-6a and H-6a'), 3.90 (dd, 1 H, *J* = 8.8, 8.8 Hz, H-2'), 3.73 (m, 1 H, H-5'), 3.52 (d, 1 H, *J* = 9.6 Hz, H-4), 3.42 (m, 1 H, H-5), 2.09 (s, 3 H, CH_3CO), 2.06 (s, 6 H, 2 x CH_3CO), 2.04, 1.99, 1.98 and 1.91 (4 s, 12 H, 3 x CH_3CO and $CH_3C{=}N$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.1, 170.7, 170.4, 169.3 and 169.2 (6 x C=O), 166.7 ($CH_3C{=}N$) 102.3 and 99.1 (C-1 and C-1'), 76.7, 72.8, 71.9, 70.4, 68.4, 67.7, 64.9, 63.1, 62.0 and 54.4 (2 x CH_2O and 8 x CH), 23.0, 20.9, 20.8, 20.7, 20.6 and 20.5 (6 x CH_3CO), 13.9 ($CH_3C{=}N$); HRMS (EI) calcd for $C_{25}H_{33}N_2O_{15}$ $(M - CH_3)^+$ 601.1881, found 601.1883 and for $C_{24}H_{33}N_2O_{13}$ $(M - CH_3CO_2)^+$ 557.1982, found 557.1986.

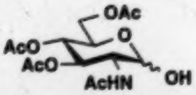


Methyl 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2'-acetamido-3',4',6'-tri-*O*-acetyl-2'-deoxy-β-D-glucopyranosyl)-β-glucopyranoside (105). To a mixture of oxazoline **104** (50.0 mg, 0.081 mmol) and dry 4 Å molecular sieves (80 mg) in dry 1,2-dichloroethane (2 mL) was added trimethylsilyl trifluoromethanesulfonate (3.20 μL, 16.2 μmol) and stirring was continued for 15 min. Dry methanol (13.0 mg, 0.406 mmol) was added and the mixture was stirred at 50 °C for 10 h. After the mixture was cooled, additional trimethylsilyl trifluoromethanesulfonate (16.0 μL, 0.081 mmol) and methanol (26.0 mg, 0.812 mmol) were added and heating was resumed for an additional 10 h. Triethylamine was then added to the cooled mixture to neutralize it. Filtration and concentration *in vacuo* gave a residue which was purified by flash column chromatography (SiO_2, CH_2Cl_2-CH_3OH, 20:1) to afford **105** (36.4 mg, 70 %) as a white solid: mp 278-280 °C (lit.[252] mp 282-284 °C); $[\alpha]_D$ -55.9° (*c* 0.30, $CHCl_3$)]; IR ($CHCl_3$ cast) 3292, 2939, 1746, 1660, 1546, 1373 cm^{-1}; ^1H NMR ($CDCl_3$ + CD_3OD, 360 MHz) δ 5.06 (t, 1 H, *J* = 9.5 Hz, H-3'), 4.93 (t, 1 H, *J* = 8.4 Hz, H-3), 4.89 (t, 1 H, *J* = 9.5 Hz, H-4'), 4.46 (d, 1 H, *J* = 8.4 Hz, H-1'), 4.31-4.24 (m, 3 H, H-1, H-6'a + H-6'b), 4.03-3.84

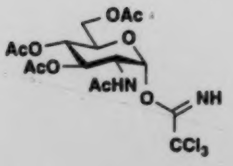
(m, 1 H, H-6a + H-6b) 3.81 (dd, 1 H, J = 8.4, 9.7 Hz, H-2'), 3.76 (m, 1 H, H-2), 3.71-3.50 (m, 3 H, H-4, H-5 and H-5') 3.34 (s, 3 H, OCH$_3$), 2.01, 1.96, 1.92, 1.89, 1.87, 1.81 and 1.79 (7 s, 21 H, 7 x CH$_3$CO); ^{13}C NMR ($CDCl_3$ + CD_3OD, 100 MHz) δ 171.4, 171.1,170.7, 170.6, 170.3, 169.9 and 169.6 (7 x C=O), 101.4 and 100.7 (C-1 and C-1'), 75.9, 73.0, 72.4, 72.3, 71.4, 68.1, 56.4, 54.2 and 53.3 (8 x CH and OCH$_3$), 62.5 and 61.6 (2 x CH$_2$O), 22.4, 22.3, 20.5, 20.3, 20.2 and 20.1 (7 x CH$_3$CO); HRMS (ES) Calcd for $C_{27}H_{40}N_2O_{16}Na$ (MNa$^+$) 671.2276, found 671.2279 and for $C_{27}H_{41}N_2O_{16}$ (MH$^+$) 649.2456, found 649.2477. Anal. Calcd for $C_{27}H_{40}N_2O_{16}$: C, 50.00; H, 6.22; N, 4.32. Found, C; 49.60; H, 5.86; N, 4.12.



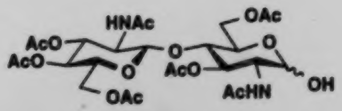
3-Bromopropyl 2-Acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2'-acetamido-3',4',6'-tri-*O*-acetyl-2'-deoxy-β-D-glucopyranosyl)-β-glucopyranoside (106). Freshly distilled 3-bromopropanol (3.5 mL, 3.9 mmol) was added to a mixture of oxazoline **104** (481 mg, 0.780 mmol), azeotropically dried 10-(*R*)-camphor sulphonic acid (72 mg, 0.312 mmol) and powdered 4Å molecular sieves (770 mg) in dry 1,2-dichloroethane (60 mL). The resulting mixture was stirred at 70 °C for 26 h, then cooled to room temerature, washed with water and dried (Na_2SO_4). The residue obtained following evaporation of the solvent *in vacuo* was purified by flash column chromatography (SiO_2, CH_2Cl_2-MeOH, 20:1) to give **106** (550 mg, 93 %) as a white solid: mp 231 °C (dec.); $[\alpha]_D^{20}$ -30.2° (*c* 0.51, CH_2Cl_2); IR ($CHCl_3$ cast) 3281, 3086, 1744, 1662, 1547, 1433 cm^{-1}; ^{1}H NMR (CD_2Cl_2, 360 MHz) δ 6.08 (d, 1 H, J = 8.9 Hz, NH'), 5.85 (d, 1 H, J = 9.4 Hz, NH), 5.21 (t, 1 H, J = 9.5 Hz, H-3'), 5.10 (dd, 1 H, J = 10.0, 8.5 Hz, H-3), 5.02 (t, 1 H, J = 9.6 Hz, H-4'), 4.59 (d, 1 H, J = 8.4 Hz H-1), 4.46 (d, 1 H, J = 8.1 Hz, H-1'), 4.39-4.34 (m, 2 H, H-6'a and H-6'b), 4.26 (dd, 1 H, J = 12.0, 5.0 Hz, H-6a), 4.02 (dd, 1 H, J = 12.4, 2.2 Hz, H-6b), 3.95-3.87 (m, 2 H, H-2 and H-1"a), 3.82-3.59 (m, 5 H, H-2', H-4, H-1"b, H-3"a and H-3"b), 3.48 (dd, 2 H, *J* = 6.9, 5.7 Hz, H-5 and H-5'), 2.17-1.96 (m, 2 H, H-2"a and H-2"b), 2.12, 2.05, 2.04, 1.99, 1.98, 1.92 and 1.91 (7 s, 21 H, 7 x CH_3CO); ^{13}C NMR (CD_2Cl_2, 100 MHz) δ 171.3, 171.1, 171.0, 170.8, 170.7, 170.4 and 169.7, (7 x C=O), 101.9 and 101.4 (C-1 and C-1'), 76.3, 73.3, 72.7, 72.6, 72.3, 68.5, 55.2 and 54.3 (8 x CH), 67.7, 62.6 and 62.1 (3 x CH_2O), 32.8 and 30.9 (2 x CH_2), 23.4, 23.3, 21.2, 21.0, 20.9 and 20.8 (7 x CH_3CO); HRMS (EI) Calcd for $C_{29}H_{44}N_2O_{16}{}^{79}Br$ 755.1874, found 755.1865; Anal. Calcd for $C_{29}H_{44}N_2O_{16}Br$: C 46.10; H 5.74; N 3.71. Found: C 46.08; H 5.94; N 3.66.



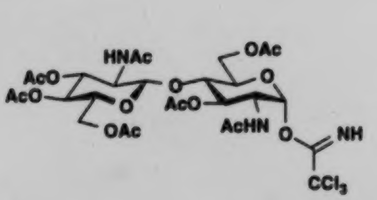
2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucose (107). To a solution of pentacetyl-α-glucosamine **101** (250 mg, 0.642 mmol) in dry THF (5 mL) was added dry benzylamine (73.0 mg, 0.681 mmol) drop-wise and the mixture was stirred at room temperature. After 3 days the reaction mixture was concentrated to give a yellow syrup which was purified by flash column chromatography (SiO_2, hexane-ethyl acetate, gradient, 70% to 100% ethyl acetate) to give **107**[267] (189 mg, 85 %) as a white foam: IR ($CHCl_3$ cast) 3360, 1746, 1659, 1538, 1433, 1368 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.92 (d, 1 H, *J* = 9.3 Hz), 5.31-5.24 (m, 2 H), 5.12 (t, 1 H, *J* = 9.6 Hz), 4.31-4.08 (m, 4 H), 2.09, 2.03, 2.02 and 1.96 (4 s, 12 H, 4 x CH_3CO); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.4, 170.9, 170.4 and 169.4 (4 x CH_3CO), 91.6 (C-1), 70.9, 68.2, 67.5 and 52.3 (4 x CH), 62.1 (CH_2O), 23.1, 20.7 and 20.6 (4 x CH_3CO); MS (ES) *m/z* (relative intensity) 370.1 (MNa$^+$, 13%), 348.1 (MH$^+$, 100), 330.1 (MH$^+$ -H_2O, 55); Anal. Calcd for $C_{14}H_{21}NO_9$: C, 48.41; H, 6.09; N, 4.03. Found: C, 48.39; H, 6.35; N, 3.92.



2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyltrichloroacetimidate (108).[268] To a solution of **107** (51.7 mg, 0.149 mmol) in CH_2Cl_2 (1 mL) was added freshly distilled trichloroacetonitrile (430 mg, 2.98 mmol) and DBU (11.3 mg, 0.0744 mmol) at 0 °C and the reaction was followed by tlc. After 1 h the reaction mixture was concentrated *in vacuo* (< 30 °C) and the residue obtained was purified by flash column chromatography (SiO_2, petrolum ether-ethyl acetate, gradient, 1:1 to 2:1) to give **108** (53 mg, 73 %) as a white foam: IR ($CHCl_3$ cast) 3332, 3295, 1744, 1670, 1543 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 8.79 (s, 1 H, C=N$\underline{H}$), 6.33 (d, 1 H, *J* = 3.6 Hz, $\underline{H}$-1), 5.68 (d, 1 H, *J* = 8.8 Hz, N-$\underline{H}$), 5.31-5.19 (m, 2H), 4.55-4.49 (m, 1 H), 4.22 (dd, 1 H, *J* = 12.9, 4.6 Hz, $\underline{H}$-6a), 4.11-4.06 (m, 2 H, $\underline{H}$-6b, $\underline{H}$-5), 2.05, 2.03, 2.02 and 1.91 (4 s, 12 H, 4 x $C\underline{H}_3CO$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.5, 170.5, 170.0 and 169.1 (4 x $CH_3\underline{C}O$), 160.2 ($\underline{C}$=NH), 94.7 ($\underline{C}$-1), 90.7 ($\underline{C}Cl_3$), 70.6, 70.2, 67.3 and 51.7 (4 x $\underline{C}H$), 61.4 ($\underline{C}H_2O$), 22.9, 20.6, 20.5 and 20.4 (4 x $\underline{C}H_3CO$); MS (ES) *m/z* (relative intensity) 370 (MH - (2 Ac and HCl))$^+$, 100%).

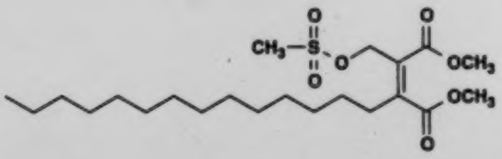


2-Acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2'-acetamido-3',4',6'-tri-*O*-acetyl-2'-deoxy-β-D-glucopyranosyl)-α-glucopyranose (110).[269] To a solution of chitobiose hexaacetate **103** (2.00 g, 2.96 mmol) in dry DMF (16 mL) was added hydrazine acetate (327 mg, 3.55 mmol) and the mixture was stirred at room temperature for 1 h. The reaction mixture was then diluted with ethyl acetate (80 mL) and washed with H_2O (2 x 40 mL). The aqueous layer was extracted with ethyl acetate (3 x 100 mL) and the combined organic extracts were washed with a saturated solution of $NaHCO_3$ (60 mL) and dried (Na_2SO_4) to give a white solid. Purification by flash column chromatography (SiO_2, CH_2Cl_2-CH_3OH, gradient, 30:1 to 10:1) gave the desired product **110** (1.3 g, 69 %) as as amorphous powder: IR ($CHCl_3$ cast) 3575, 3363, 2957, 2496, 1744, 1662, 1540, 1435 cm^{-1}; ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 5.28 (dd, 1 H, *J* = 10.8, 9.2 Hz, H-3), 5.01 (dd, 1 H, *J* = 10.2, 9.4 Hz, H-3'), 4.96 (d, 1 H, *J* = 3.5 Hz, H-1), 4.86 (t, 1 H, *J* = 9.8 Hz, H-4'), 4.31 (d, 1 H, *J* = 8.4 Hz, H-1'), 4.27 (dd, 1 H, *J* = 12.5, 4.2 Hz, H-6'a), 4.18 (d, 1 H, *J* = 9.9 Hz, H-2), 4.05-3.85 (m, 4 H, H-4, H-6a, H-6b, and H-6'b), 3.77 (dd, 1 H, *J* = 10.5, 8.4 Hz, H-2'), 3.57-3.51 (m, 2H, H-5 and H-5'); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 171.9, 171.5, 171.4, 170.9, 170.7, 170.6 and 170.5 (7 x C=O), 100.9 and 90.9 (C-1 and C-1'), 75.9, 72.0, 71.2, 71.1, 68.1, 67.7, 54.0 and 52.0 (8 x CH), 62.5 and 61.5 (2 x CH_2O), 22.2, 22.1, 20.4, 20.3, 20.2, 20.1 and 20.0 (7 x CH_3CO); HRMS (ES) Calcd for $C_{26}H_{38}N_2O_{16}Na$ (MNa$^+$) 657.2119, found 657.2124 and for $C_{26}H_{39}N_2O_{16}$ (MH$^+$) 635.2299, found 635.2296. Anal. Calcd for $C_{26}H_{38}N_2O_{16}$: C, 49.21; H, 6.04; N, 4.41. Found: C, 49.58; H, 6.00; N, 4.21.



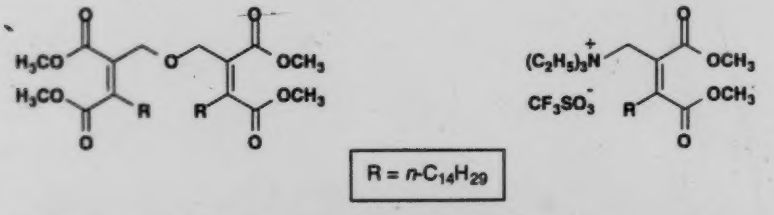
2-Acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2'-acetamido-3',4',6'-tri-*O*-acetyl-2'-deoxy-β-D-glucopyranosyl)-α-glucopyranose trichloroacetimidate (111). To a solution of **110** (228 mg, 0.359 mmol) in CH_2Cl_2 (12 mL) was added freshly distilled trichloroacetonitrile (1.04 g, 7.17 mmol) and DBU (27.3 mg, 0.179 mmol) at 5 °C and the reaction was stirred for 1 h. Concentration *in vacuo* (< 30 °C) gave a yellow foam which was purified by flash column chromatography (SiO_2, ethyl acetate-methanol, 99:1) to afford **111** (185 mg, 66 %) as a white foam: $[\alpha]_D$ +2.7 °C (*c* 0.4, $CHCl_3$); IR ($CHCl_3$ cast) 3330, 3298, 1746, 1674, 1540, 1369 cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 8.75 (s, 1 H, C=NH), 6.26 (d, 1 H, *J* = 3.7 Hz, H-1), 6.1 (d, 1 H, *J* = 8.8 Hz, NH'), 5.75 (d, 1 H, *J*

= 8.9 Hz, NH), 5.23 (t, 2 H, J = 9.6 Hz, H-3 and H-3'), 5.03 (t, 1 H, J = 9.8 Hz, H-4'), 4.66 (d, 1 H, J = 8.3 Hz, H-1'), 4.45-4.33 (m, 3 H), 4.26 (m, 2 H), 4.03-3.98 (m, 2 H), 3.84-3.74 (m, 2 H, H-4 and H-5'), 3.65 (m, 1 H, H-5), 2.11, 2.07, 2.06, 2.00, 1.99, 1.92 and 1.91 (7 s, 21 H, 7 x CH_3CO); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.3, 171.0, 170.7, 170.5, 170.2, 170.0 and 169.4 (7 x C=O), 160.5 (C=NH), 101.1 and 94.7 (C-1 and C-1'), 90.8 (CCl_3), 75.7, 72.3, 71.9, 71.3, 70.6, 68.2, 55.1 and 51.8 (8 x CH), 61.8 and 61.6 (2 x CH_2O), 23.2, 23.0, 20.8, 20.7, 20.6, 20.5 and 20.4 (7 x CH_3CO); HRMS (ES) Calcd for $C_{28}H_{38}Cl_3N_3O_{16}Na$ (MNa^+) 800.1215, found 800.1223.



Dimethyl (*Z*)-2-methanesulfonylmethyl-3-tetradecylbutenedioate (112). To a solution of alcohol **89** (50 mg, 0.135 mol) in CH_2Cl_2 (2 mL) was added triethylamine (18.0 mg, 0.180 mmol) and methanesulfonyl chloride (19.0 mg, 0.162 mmol) at - 50 °C. The mixture was stirred at the same temperature for 10 min and then warmed to 0 °C and stirred for another 10 min before it was poured into ice-cold H_2O (3 mL). Extraction with CH_2Cl_2 (4 x 5 mL), drying (Na_2SO_4) and evaporation of the solvent *in vacuo* gave mesylate **112** (57 mg, 94 %) as a white solid: mp 46-47 °C; IR (CH_2Cl_2 cast) 2918, 2849, 1733, 1725, 1644, 1468 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 4.98 (s, 2 H, OCH_2C=C), 3.80 and 3.77 (2s, 6 H, 2 x OCH_3), 3.02 (s, 3 H, CH_3SO_3), 2.48 (t, 2 H, J = 7.6 Hz, -CH_2CH_2C=C), 1.45 (qn, 2 H, -CH_2CH_2C=C), 1.32-1.21 (m, 22 H, -$(CH_2)_{11}$), 0.86 (t, 3 H, J = 6.6 Hz, CH_3-chain); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 168.9 and 165.4 (2 x C=O), 151.6 and 124.8 (C=C), 63.9 (OCH_2C=C), 52.7 and 52.5 (2 x OCH_3), 37.9 (CH_3SO_3), 31.9, 31.4, 29.7, 29.6, 29.4, 29.3, 29.2, 27.9 and 22.7 (-$(CH_2)_{13}$-), 14.1 (CH_3-chain); MS (FAB, Cleland) m/z (relative intensity) 471.5 (MNa^+, 5%), 449.4 (MH^+, 17%), 417.4

(100), 353.2 (30), 321.1 (64). Anal. Calcd for $C_{22}H_{40}O_7S$: C, 58.90; H, 8.99. Found: C, 58.77; H, 8.77.

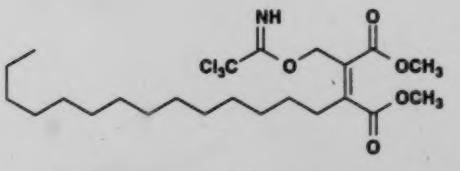


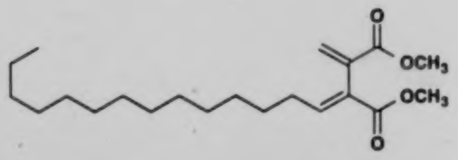
Bis(dimethyl (*Z*)-2-hydroxymethyl-3-tetradecylbutenedioate) (114) and dimethyl (*Z*)-2-triethylammonium-3-tetradecylbutenedioate triflate salt (115). To a solution of alcohol **89** (200 mg, 0.540 mmol) in CH_2Cl_2 (5 mL) at -50 °C was added triethylamine (97.8 µL, 0.702 mmol). After 5 min triflic anhydride (109 µL, 0.648 mmol) was injected into the reaction mixture and stirring was continued at -50 °C for 1 h. The pale yellow solution was allowed to warm slowly to 0 °C and then poured into a solution of cold 5% $NaHCO_3$ (10 mL) and quickly extracted with CH_2Cl_2. The organic extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give a crude product. Analysis by ^1H NMR and mass spectroscopy indicated that the major component was the salt. Purification by flash column chromatography (SiO_2, hexane-ether, 2:1) gave compound **115** (50 mg, 13 %).

For **114**: ^1H NMR ($CDCl_3$, 360 MHz) δ 4.25 (s, 2 H, $C\underline{H}_2$), 3.77 (s, 6 H, 2 x $OC\underline{H}_3$), 3.30 (q, 6 H, *J* = 7.2 Hz, $(CH_3C\underline{H}_2)_3N$), 2.54 (t, 2 H, *J* = 6.9 Hz, chain-$CH_2C\underline{H}_2C{=}C$), 1.35 (t, 9 H, *J* = 7.1 Hz, $(C\underline{H}_3CH_2)_3N$), 1.30-1.17 (br m, 24 H, $-(CH_2)_{12}-$), 0.85 (t, 3 H, *J* = 6.9 Hz, $C\underline{H}_3$-chain); MS (ES) *m/z* (relative intensity) 454.3 (MNa^+, 100%).

For **115**: IR ($CHCl_3$ cast) 2950, 2924, 2853, 1729, 1643, 1434 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.27 (s, 4 H, 2 x $-C\underline{H}_2O$), 3.76 and 3.74 (2s, 12 H, 4 $C\underline{H}_3O$), 2.40 (t, 4 H, *J* = 7.2 Hz, chain-$CH_2C\underline{H}_2C{=}C$), 1.43-1.20 (m, 48 H, 2 x $-(C\underline{H}_2)_{12}$), 0.87 (t, 3 H, *J* = 6.5 Hz, $C\underline{H}_3$-chain); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.1 and 167.4 (4 x $\underline{C}{=}O$), 144.7

and 131.6 (2 x $\underline{C}=\underline{C}$), 65.9 (2 x $\underline{C}H_2O$), 52.3 and 52.2 (4 x $O\underline{C}H_3$), 31.9, 30.4, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.2 (2 x $-(\underline{C}H_2)_{13}$), 14.1 (2 x $\underline{C}H_3$-chain); HRMS (EI) Calcd for $C_{21}H_{37}O_5$ (M+ - $C_{21}H_{37}O_4$) 369.2641, found 369.2638. MS (ES) *m/z* (relative intensity) 745.4 (MNa^+, 100%).





Dimethyl (*Z*)-2-trichloroacetimidatyloxymethyl-3-tetradecylbutenedioate (119) and 1,3-dicarbomethoxyheptadec-1,3-diene (120). To a solution of alcohol **89** (200 mg, 0.539 mmol) in CH_2Cl_2 (5 mL) was added freshly distilled trichloroacetonitrile (1.22 g, 8.46 mmol) and DBU (32.3 mg, 0.212 mmol) at 0 °C and the reaction was monitored by tlc (petroleum ether-ether, 1:1). The reaction was complete in 15 min and the mixture was concentrated *in vacuo* to give 345 mg of a brown residue. Purification by flash column chromatography (SiO_2, petrolum ether-ether, 3:1) gave **119** (220 mg, 79 %) along with the by-product **120** (14 mg, 7 %) both as colorless oils.

For **119**: IR ($CHCl_3$ cast) 3345, 2920, 2850, 1731, 1666, 1466, 1434 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 8.39 (s, 1 H, $C=N\underline{H}$), 5.04 (s, 2 H, $OC\underline{H}_2C=C$), 3.79 and 3.74 (2s, 6 H, 2 x $OC\underline{H}_3$), 2.47 (t, 2 H, J = 7.7 Hz, $-CH_2C\underline{H}_2C=C$), 1.45 (qn, 2 H, $-C\underline{H}_2CH_2C=C$), 1.36-1.10 (m, 22 H, $-(C\underline{H}_2)_{11}-$), 0.86 (t, 3 H, J = 6.6 Hz, $C\underline{H}_3$-chain); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.2 and 166.2 (2 x $\underline{C}=O$), 162.2 ($\underline{C}=NH$), 148.3, 127.5 ($\underline{C}=\underline{C}$), 90.9 ($\underline{C}Cl_3$), 64.0 ($O\underline{C}H_2C=C$), 52.3 (2 x $O\underline{C}H_3$), 31.8, 31.0, 29.6, 29.5, 29.4, 29.3, 29.2, 28.0 and 22.6 ($-(\underline{C}H_2)_{13}-$), 14.0 ($\underline{C}H_3$-chain); HRMS (EI) Calcd for $C_{23}H_{38}Cl_3NO_5$ 513.1816, found 513.1808 and for $C_{21}H_{35}Cl_3NO_3$ (M^+ - $C_2H_3O_2$) 454.1682, found 454.1686. Anal. Calcd for $C_{23}H_{38}Cl_3NO_5$: C, 53.65; H, 7.44; N, 2.72. Found: C, 53.62; H, 7.48; N, 2.73.

For **120**: IR ($CHCl_3$ cast) 2924, 1853, 1731, 1434 cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 6.22 (t, 1 H, *J* = 7.6 Hz, $-CH_2C\underline{H}=C$), 6.18 (d, 1 H, *J* = 1.4 Hz, $\underline{H}HC=CCO$), 5.71 (d, 1 H, *J* = 1.3 Hz, $H\underline{H}C=CCO$), 3.74 and 3.71 (2s, 6 H, 2 x $OC\underline{H}_3$), 2.54 (q, 2 H, *J* = 7.4 Hz, $-CH_2C\underline{H}_2CH=C$), 1.46 (qn, 2 H, $-C\underline{H}_2CH_2CH=C$), 1.34-1.23 (m, 20 H, $-(C\underline{H}_2)_{10}-$), 0.88 (t, 3 H, *J* = 6.5 Hz, $C\underline{H}_3$-chain); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.7 and 166.5 (2 x $\underline{C}=O$), 146.7 ($\underline{C}H=C$), 140.4, 130.3 ($H_2C=\underline{C}$ and $\underline{C}=CH$), 126.5 ($H_2\underline{C}=C$), 52.1 and 51.4 (2 x $O\underline{C}H_3$), 31.9, 29.6, 29.5, 29.4, 29.3, 29.1 and 22.7 ($-(\underline{C}H_2)_{12}-$), 14.2 ($\underline{C}H_3$-chain); HRMS (EI) Calcd for $C_{21}H_{36}O_4$ 352.2614, found 352.2616, and for $C_{20}H_{32}O_3$ ($M^+ - CH_4O$, 100 %) 320.2351, found 320.2354.

CHAPTER 5 References

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